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ECBC-TR-517

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGY ASSESSMENT

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14. ABSTRACT
The U.S. Army Center for Environmental Health Research, with support from Army client organizations and funding from Army Science and Technology Objective (STO) IV.ME.2004.03, is developing an Environmental Sentinel Biomonitor (ESB) system to provide rapid toxicity identification for a broad spectrum of chemicals in water. A critical initial phase of the STO is to test and evaluate toxicity sensor technologies (also called ESB system technologies). Because there are a number of potentially feasible technologies that could meet the goals of the ESB program, a downselect was performed to evaluate these technologies and select the most promising technologies for further development as part of an ESB system. The methodology and process to complete the downselect was developed with user representatives and technology experts. The methods and processes used produce repeatable, defensible, and justifiable investment decisions.

15. SUBJECT TERMS

Water monitoring	Multi-attribute utility model	Short-term military model
Concept of use	Decision analysis	Downselect
Human lethal dose	Threshold requirement	Toxic industrial chemicals
Detection of rapidity of response and sensitivity		

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PREFACE

The work described in this report was authorized under Army Science and Technology Objective (STO) IV.ME.2004.03. The work described in this report was started in January 2004 and completed in September 2005.

This final report was prepared in response to a request from the U.S. Army Center for Environmental Health Research to develop a methodology to evaluate Environmental Sentinel Biomonitor (ESB) technologies and then to evaluate and downselect the most appropriate ESB technologies to further develop into an ESB system.

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ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM
TECHNOLOGY ASSESSMENT

1. OVERVIEW

The Environmental Sentinel Biomonitor (ESB) system downselection process was conducted in FY04 and FY05. An initial technical report described work completed in FY04 and the plan to complete the project in FY05.* Information in this report takes precedence over the initial technical report and describes the methodology and results for the entire project's effort.

1.1 Introduction.

The U.S. Army Center for Environmental Health Research (USACEHR), with support from Army client organizations and funding from Army Technology Objective (ATO) IV.MD.2004.03, is developing an ESB system to provide rapid toxicity identification for a broad spectrum of chemicals in water. The focus of the ESB system is to detect toxicity associated with non-militarized chemicals (i.e., toxic industrial chemicals [TICs] and toxic industrial materials [TIMs]) in water.

A critical initial phase of the ATO is to test and evaluate ESB technologies. Because there are a number of potentially feasible technologies that could meet the goals of the ESB program, a downselect was performed to evaluate these technologies and select the most promising ones for further development as part of an ESB system.

1.2 Background.

Deployed U.S. forces face the possibility of drinking water exposed to a wide range of toxic industrial or agricultural chemicals as a result of normal use (e.g., farm run-off), damaged infrastructures, accidental spills, or deliberate chemical contamination of water. Unfortunately, rapid detection capabilities for toxic chemicals in water are limited and may not provide sufficient warning of developing toxic hazards.

The ESB system is intended to monitor responses of biological components (e.g., enzymes, cells, tissues, or whole organisms) exposed to water and to provide rapid responses/warnings should toxic conditions develop. Cell-based sensors are becoming available that integrate biological systems with electronic monitoring, facilitating rapid response to developing toxicity in water using very small systems.

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* Kooistra, S.; Walther, J.; *Initial Technology Assessment For The Environmental Sentinel Biomonitor (ESB) System*; ECBC-TR-477; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2006; UNCLASSIFIED Report (AD-A454 235).

1.3 ESB Program Objective.

The ESB program will incorporate current toxicity sensor technologies into an ESB system having size, weight, and logistical characteristics suitable for a range of Army requirements that will complement current chemical monitoring systems and provide rapid toxicity identification for a broad spectrum of chemicals in water. The optimal system may be a complementary set of toxicity sensors, which would provide:

- Rapid response. Required response times may range from a few minutes to an hour depending upon the particular Army use scenario.
- Sensitivity. One ESB technology may not adequately detect all TICs/TIMs. The assessment will consider not only which ESB technology provides the best overall response to the test chemicals, but also which set of technologies can complement each other by filling gaps in toxicity response for individual technologies as well as providing mutual confirmation of a toxic response.

1.4 Assessment Process Overview.

A decision analysis-based methodology was developed to conduct the ESB system downselection. Decision analysis is a structured process for decision-making based on established principles of operations research. The decision analysis process includes systematic development and examination of alternative courses of action to define and clarify available choices and associated advantages and disadvantages. It also includes thorough documentation of results and associated rationale so that final recommendations can be readily explained and defended. The study consisted of six phases (described in sections 2.1-2.6).

1. Form study/assessment team.
2. Develop ESB system applications (i.e., scenarios).
3. Define technical requirements of ESB system for each application.
4. Identify candidate ESB technologies. Develop and use a screening model to reduce number of technologies to consider. Collect missing toxicity response data.
5. Develop quantitative assessment models.
6. Generate and analyze results using quantitative assessment model.

2. ASSESSMENT PROCESS AND RESULTS

2.1 Study/Assessment Team Formed.

An assessment team was formed in early FY04 to conduct the downselect. The team was led by Dr. William van der Schalie (USACEHR) and Dr. Thomas Gargan II (USACEHR), with support from the Decision Analysis Team (DAT) of the Edgewood Chemical and Biological Center. The assessment team was made up of Army user representatives (members of an Integrated Product Team) and technical experts from collateral organizations and academia (see Appendix A for a list of team members and their roles).

The role of the user representatives was to articulate the applications/scenarios for all possible Army users of an ESB system. They also defined the technical requirements for the ESB system for each scenario. Their final task was to help develop the quantitative downselection assessment model.

The role of the technical expert was to be knowledgeable about the ESB technologies, help develop the assessment models, and then assess the technologies against the models. Although the user representatives have primary responsibility for model development, the technical expert's input was important because they provided input on the technical feasibility of measures for the models.

2.2 ESB System Applications/Scenarios Defined.

Several Army applications for an ESB system were identified early in the ATO program process. These include:

- Use in conjunction with the Tactical Water Purification System (TWPS) and Light Weight Water Purification System, including assessment of water pre- and post-treatment and supporting decisions regarding the suitability of using a freshwater bypass system. Use of an ESB system is consistent with a recommendation in the Pre-Planned Product Improvement section of the TWPS Operational Requirements Document.
- Testing water produced by on-board water generation equipment in Manned Ground Vehicles being developed under the Future Combat Systems program.
- Use by Preventive Medicine personnel to evaluate potable water quality in a variety of situations.
- Use at Army installations, both domestically and overseas, to evaluate source and drinking water quality.

The user representatives reviewed the above Army applications and identified others. They then analyzed and grouped these applications to form the fewest number of scenarios that would represent all identified Army applications. This was done through an iterative process and resulted in four basic scenarios to represent the situations in which the Army operates. Two of the four basic scenarios were further subdivided by type of water tested and collection method to create two more scenarios, for a total of six.

The type of water tested could either be raw (i.e., water that has not been treated with any mechanism [e.g., filters] or chemicals [e.g., chlorine]) or treated (e.g., water treated with a TWPS, hand-held water treatment device, or chemicals).

There are two types of collection methods. In the "continuous flow" method, for example, the water must be continually tested as the water flows inside a pipeline from one point to the next. An ESB system using the continuous flow method must constantly monitor the water for contamination. The results of the tests need to be available in near real-time to prevent use or contamination further along in the distribution system. The "grab sample" method is

defined as procuring water from a source and then testing it off-line. The water needs to be collected and tested before it is used, with maximum response times ranging between a few minutes to an hour.

The six scenarios are presented as an overview in *Figure 1* and further described in the rest of section 2.2.1. *Figure 1* briefly describes each scenario and notes visually with gray boxes the sample collect methods and type of water the ESB technology would test. For example, for the Individual/Small Team scenario the sample collection method is a grab sample and the type of water tested is both raw and treated.

<table>
<tr><th></th><th></th><th colspan="2">Sample Collection Method</th><th colspan="2">Type of Water Tested</th></tr>
<tr><th>Six Scenarios Developed</th><th>Scenario Overview</th><th>Grab</th><th>Contin-uous</th><th>Raw Water</th><th>Treated Water</th></tr>
<tr><td>Individual/ Small Team</td><td>ESB system would be transported and operated by each individual.</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Small Unit</td><td>ESB system would be transported by a vehicle, which has limited capacity to carry an ESB system. ESB system would be operated by anyone within team.</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Field/ Contingency (grab sample, raw or treated water)</td><td rowspan="2">ESB system would be transported by vehicle (e.g., truck, C-130) with significant more capacity to carry an ESB system than in the Individual/Small Team and Small Unit scenarios. The ESB system can be larger and heavier. ESB system would be operated by personnel of higher grades and/or skills so a more complicated ESB system than in the Individual/Small Team and Small Unit scenario could be operated/used.</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Field/ Contingency (continuous sample, treated water)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Fixed Base (grab sample, raw or treated water)</td><td rowspan="2">ESB system can be larger and heavier than all other scenarios because of the options available to transport an ESB system to an existing/permanent base or to a rear area in a deployed situation. ESB system could be operated/used by personnel of even higher grades and/or skills than all other scenarios and thus be a more complicated ESB system than all other scenarios.</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Fixed Base (continuous sample, raw or treated water)</td><td></td><td></td><td></td><td></td></tr>
</table>

Figure 1: Overview of Six Scenarios Developed

2.1 Individual/Small Team Scenario.

This scenario represents one Soldier or a small team of Soldiers operating motely away from a fixed or field/contingency base. In this scenario each Soldier can operate dependently because they have their own ESB system. An example is a small team of Soldiers erating remotely in the hills of Afghanistan – Soldiers are flown in and out of this remote cation every 14 days; they must procure local water each day.

The ESB system provides the individual Soldier or small team the ability to test a ab sample of raw or treated water in the field for the presence of toxic chemicals that could use acute health effects and degrade battlefield readiness/performance. The Soldier could test w water from a surface water source prior to disinfecting and drinking it or test water that has en treated using a hand-held water treatment device (e.g., Army's Light Weight Water rification System). This gives the individual Soldier an enhanced capability to check the ality of his personally acquired/produced water before consuming it. The ESB system would used in emergency situations when the Soldier does not have access to a doctrinally treated d/or distributed water supply and must acquire, produce, or purify his/her own water (e.g., itial entry, remote site, and Special Operations Forces missions).

2.2 Small Unit Scenario.

This scenario is similar to the individual/small team scenario. Instead of an dividual operating with only what they can carry or procure locally (e.g., local untreated ater), the Soldiers are operating within a small unit construct. The ESB system would be insported within a vehicle. Water could be procured the same way as with the dividual/Small Team scenario or through water produced from the Future Combat Systems – anned Ground Vehicle (MGV). The MGV has the ability to generate potable water through a ocess that starts with capturing the exhaust of the vehicle. The purpose of the ESB system in is situation would be to independently verify that the water produced by the MGV is potable.

2.3 Field/Contingency Scenarios.

There are two scenarios, differing only in the type of water tested and collection ethod, which represent a field/contingency operation. In the Field/Contingency scenarios ore than a small team or unit of Soldiers is living and working, for more than 15 days, and their ater is procured from a questionable source (quality/safety of water is not known). The first enario collects water with a grab sample and tests both raw and treated water. An example of first scenario is a reconnaissance team, in its mission to find a suitable source of water for the verse Osmosis Water Purification Unit (ROWPU) or TWPS units, must test existing water urces in the area of the field/contingency base. Another example is Preventive Medicine rsonnel need to periodically check for TICs/TIMs in storage tanks and distribution systems. e objective is to rapidly identify accidental or intentional spills, discharges or contamination.

The second scenario collects water with a continuous flow sample and tests only ated water. An example of the second scenario is quartermaster personnel using a continuous

flow ESB system to monitor both water that is the source for their ROWPU or TWPS as well as the water produced by these units/systems.

2.2.4 Fixed Base Scenarios.

There are two scenarios, differing only by type of water tested and collection method, which represent operations at a permanent base either within the United States or overseas or at rear areas in deployed situations. In these scenarios, the Soldiers may obtain their drinking water from water treatment plants that meet U.S. quality standards (potable water is not being created with a ROWPU or TWPS). The water treatment plant could be located either on base or off base, with the water piped in. The population will be less homogeneous than the other scenarios if dependents are using the water supply. An example of the first scenario, grab Sample to Test Raw or Treated Water, is Public Works, Preventive Medicine, and possibly Security personnel who must perform spot checks of water system assets (e.g., treatment plant product water, individual storage tanks, distribution system) for the presence of TICs/TIMs at levels that could cause harmful health effects. An example of the second scenario, continuous flow sample to test raw or treated water, is installation Public Works personnel who must monitor continuously for the introduction of TICs/TIMs into the water supply system in order to prevent or minimize problems.

2.3 Technical Requirements Defined.

The user representatives developed technical requirements for each of the six scenarios. These requirements define in detail the performance specifications for the ESB system and are made up of 26 questions (covering eight assessment categories) and their answers (see Appendix B). The purpose of this document is to provide more specific information for each of the six scenarios and to help the technical experts know what information must be collected (through research or additional testing) for the downselection evaluation. The document is not intended to be the final design specification for an ESB system. The user representatives noted both threshold/minimum requirements and ideal/preferred requirements.

Selected and Defined Target Detection Range.

The concentration range over which ESB technologies must detect TICs/TIMs (upper and lower limit) needed to be specified before the technologies could be evaluated. The user representatives agreed the threshold requirement for the ESB system technologies is to detect TICs/TIMs between the short-term Military Exposure Guidelines (MEGs) level (standard is based on a 70 kg Soldier drinking 15 liters/day for 7-14 days; see Appendix C for additional information about MEGs) and the human lethal concentration (HLC), which assumes consumption of 15 liters in one day by a 70 kg Soldier. The ESB program will not focus on detecting TICs/TIMs at more sensitive levels (e.g., long-term MEG or U.S. Environmental Protection Agency measures where the focus is on a chronic effects level) until more promising ESB technologies become available. Until then, existing analytical chemistry tools will be used to detect TICs/TIMs at chronic effect levels.

The user representatives noted that ESB technologies should detect toxicity closer to the short-term MEG level than the HLC level and that the minimum detection level must be below the HLC. Finally, user representatives stated that detecting toxicity below the short-term MEG level is not desirable because this would result in false positive readings.

2.4 Candidate ESB Technologies Identified, Technologies Eliminated with Screening Model, and Missing Data Collected.

After extensive research, 40 possible ESB system technologies were identified (listed in Appendix D) for further research and evaluation. Fact sheets were created for each technology (see Appendix E for an example) and presented to the technical experts. The information within the fact sheets encompassed performance, operational, and logistical characteristics.

Through the research process of developing the ESB technology fact sheets it was determined there was insufficient toxicity response information for a thorough comparative assessment of the ESB technologies. To complete the final ESB technology assessment, missing toxicity response data had to be collected in order to complete the final technology evaluation. Funding and time constraints did not allow for the collection of toxicity response data for all 40 technologies.

2.4.1 Reduced ESB Technologies with a Qualitative Screening Model.

A qualitative screening assessment model was developed and used to reduce the number of ESB technologies to consider further. ESB technologies were eliminated from further consideration if the technical experts were confident the technology could not help meet the ATO.

The screening model used a two-step process. In the first step, ESB technologies were eliminated because of any reason listed below.

- Technology toxicity sensitivity is inappropriate or the technology does not provide a unique end-point (i.e., metabolic, physical response).
- Technology is redundant and inferior to another technology.
- Technology is unfeasible/not viable. It does not or is not expected to meet a minimum requirement of a user scenario.
  - -Technology takes too much time to produce an end-point.
  - -Technology requires pre-culturing (e.g., requires actively taking out and culturing organism for a long period of time).

Twenty-three of the 40 technologies were eliminated by this first step. In the final step, the 17 remaining technologies were evaluated against four high level measures, and a final ranking was generated. The four high level measures are:

- Technology provides an appropriate toxicity sensitivity response (SR) and/or provides unique information (e.g., unique end-point).

- Technology is only minimally affected by interferences (I). Interferences are turbidity, color, and ionic composition of the media.
- Technology is reliable/results are repeatable (R&R).
- Technology has an appropriate rapidity of response (RoR). A green rating is 20 minutes or less, yellow is 20-60 minutes, and red is over 60 minutes.

Figure 2 provides an example of the results from using the qualitative screening assessment model. See Appendix F for details on definitions of assessment measures and the assessment methodology and Appendix G for the results of the screening process.

SCREENING ASSESSMENT/RESULTS FOR ESB SYSTEM TECHNOLOGIES								
Technology	Biological System	Step 1 Rating (see Note 1)	Step 1 Rationale and Comments	Step 2 Rating (see Note 1 & Note 2)				Step 2 Rationale and Comments
				SR	I	R&R	RoR	
Mitoscan	Enzyme (sub-mito-chondrial particles)	Green-Go to Step 2	Mammalian system may provide some unique sensitivities.	Green	Yellow	Green	Green	SR- one of the best technologies under review; I - expected to be similar to Aquanox, but no data available; R&R- thought to be high, SMPs; RoR- range of 5-20 minutes
Note 1: See Appendix F for a detailed description of assessment measure definitions and assessment methodology for both Steps 1 and 2.								
Note 2: The list of Step 2 screening measures were: 1) sensitivity response and/or provides unique information [SR], 2) handling of interferences [I], 3) reliable/repeatable [R&R], and 4) rapidity of response [RoR].								

Figure 2: Screening Assessment Results Example

Figure 3 lists the seventeen ESB technologies that were determined to be the most promising (1 is most promising, 17 least promising) by the technical experts.

Rank	Technology
1	Eclox
2	Aquanox
3	Microtox
4	Mitoscan Electron Transfer (ETR)
5	Mitoscan Reverse Electron Transfer (RET)
6	ToxScreen Metal
7	ToxScreen Organic
8	*Sinorhizobium meliloti* Assay
9	SOS Cytosensor
10	Portable Neuronal Microelectrode Array
11	Portable Cell-based Biosensor
12	Cellsense
13	Amtox
14	Toxi-chromo Test
15	Fluotox
16	ArrayScan HCS System
17	Electric Cell-Substrate Impedance Sensing using Endothelial Cells

Figure 3: ESB Technologies Chosen with Qualitative Screening Model

For the reasons stated in *Figure 4*, six technologies were dropped from further consideration.

Technology	Reason Technology was Eliminated
Aquanox	Technical experts determined Aquanox and Eclox are almost the same in the test system used and performance and decided only one of the technologies should be further studied. With additional evaluation, Eclox was determined to be slightly better, so Aquanox was dropped from further consideration.
Portable Cell- based Biosensor	The owner of technology was unable to participate in further testing.
Cellsense	European-based technology; the owner of this technology was not responsive to a request to participate in further testing.
Amtox	Extremely costly to test; not a high enough priority but may consider testing later if funding becomes available.
Fluotox	European-based technology; the owner of this technology was not responsive to a request to participate in further testing.
Array Scan HCS System	Not a high enough priority. Funding not available.

Figure 4: Technologies Dropped from Further Consideration

2.4.2 Toxicity Response Test Plan Developed and Implemented.

After the selection of the 11 ESB technologies for additional testing was completed, a twelfth technology was identified and approved for inclusion into the study: the Hepatocyte Low Density Lipoprotein Uptake. This technology was included partly because the toxicity response tests were provided at no additional cost.

The twelve technologies listed in *Figure 5* are ones the technical experts determined were the most promising and for which toxicity response data was collected. These technologies were later assessed with the quantitative models.

As stated earlier, toxicity response results were needed to further evaluate each technology. To acquire this data for the 12 ESB technologies an experimental design and analysis Toxicity Response Test Plan was developed.

Part of this experimental plan was the requirement to identify and select a set of training/test chemicals; the technical experts did this with input from the user representatives. These chemicals were used to form a common basis for comparison of test results for the ESB technologies. Twelve chemicals were selected to be the training/test chemical set (see Appendix H for a more detailed discussion on the selection of test chemicals and the list of chemicals). Battelle Memorial Institute then used the Toxicity Response Test Plan to conduct testing and determine the toxicity response for the 12 ESB technologies. These results have been published.** Note that for the Hepatocyte Low Density Lipoprotein Uptake technology the test results were provided by the vendor and were not independently verified by Battelle.

Olson, C.; James, R.; Botsford, J.; Curtis, T.; Doherty, F.; Lush, D.; McFadden, P.; O'Shaughnessy, T.; States, S.; Shoji, R. *A Medical Research and Evaluation Facility and Studies Supporting the Medical Chemical Defense Program; Subtitle – Experimental Design, Coordination, and Comparative Analysis of Toxicity Sensor Data Study*; Battelle Memorial Institute: Columbus, OH, 2005; UNCLASSIFIED Report (AD-A434473).

ESB Technology Name	Abbreviated ESB Technology Name (used in some figures and tables)
Eclox	Eclox
Microtox	Microtox
Mitoscan Electron Transfer (ETR)	Mitoscan ETR
Mitoscan Reverse Electron Transfer (RET)	Mitoscan RET
ToxScreen Metal	ToxScreen Metal
ToxScreen Organic	ToxScreen Org
Sinorhizobium meliloti Assay	S. meliloti
SOS Cytosensor	SOS Cyto
Neuronal Microelectrode Array	Neur Microel Array
Toxi-Chromotest	Toxi-Chromotest
Electric Cell-Substrate Impedance Sensing	ECIS
Hepatocyte Low Density Lipoprotein (LDL) Uptake	Hepatocyte LDL

Figure 5: Technologies Further Tested and Evaluated

2.5 Quantitative Assessment Models Described.

Based on the Toxicity Response Test results, the technical experts determined that no ESB technology or system of technologies could meet minimal/threshold requirements for the continuous flow scenarios or the Individual Soldier/Small Team scenario. User representatives, recommended these scenarios not be considered further in this downselection process, but their requirements continue to be incorporated into the ESB system objective requirements for implementation in the future as ESB system technologies mature. Therefore, only the Fixed Base, Field/Contingency, and Small Unit scenarios (grab sample collection method only) were considered further. Three separate multi-criteria decision-making models were developed.

A decision support software tool, Logical Decisions for Windows (LDW; Logical Decisions, Inc., copyright 2000), was used to develop and document the downselection assessment models.

Assessment criteria are the core of the models. The criteria are structured as a hierarchical model and are at a level that permits discrimination between the different technologies. User representatives created assessment criteria for each model from the requirements for the three scenarios previously defined (see Appendix B). Higher-level measures categories, referred to as goals, are Performance, Operational Impact, and Logistics. Specific sub-categories of criteria (e.g., *Chemical Detection*), referred to as measures, are developed to provide the degree of discrimination needed for the technology evaluation.

Each ESB technology was assessed against these measures. Each model is comprised of ten measures. Measures are composed of definitions, performance scales, and weights. Several factors were considered when developing the assessment measures. Assessment measures need to provide differentiation between the ESB technologies, so the measures had to be relevant and discriminating. Measures also had to be independent, so that aspects measured in one measure were not repeated in another measure. It was likewise important to focus on the measures that were most critical to the analytical process.

Measures can define quantitative and/or qualitative requirements or goals. For example, the *Chemical Detection* measure was quantitative, measured in numerical units – number of chemicals detected. The *Storage Conditions Required* measure, which was qualitative, was better assessed in more subjective terms, such as "demanding (refrigeration required)."

Measure definitions and performance scales are important elements in describing a measure. Measure definitions are narrative descriptions of the measures that must be adequately and appropriately stated and clearly understood. Performance scales are the "rating scheme" used to evaluate technologies against the measure. Some performance scales may be continuous (e.g., numeric range with *Chemical Detection* measure), while others may be discontinuous, or discrete levels referred to as labels (e.g., levels of storage conditions required with *Storage Conditions Required* measure). These two examples are shown below.

Chemical Detection

Utility	Performance Scale
100	Six chemicals detected
0	No chemicals detected

Storage Conditions Required

Utility	Performance Scale
100	Easy, ambient conditions
50	Average, controlled room temperature
0	Demanding, refrigeration required

Performance scales are expressed as utility functions, which convert different measures to common units. In order to set relevant endpoints and to establish appropriate intermediate utility values, ESB technology characteristics had to be well defined. Utility values of 100 and 0 were assigned to the high and low end of each performance scale and intermediate level utilities were derived through various elicitation techniques focused on the relative importance of moving to-and-from various points on the utility function.

Figure 6 illustrates the intermediate utility points, in the form of a utility curve, for the *Test Reproducibility* measure. This utility curve is referred to as a "risk seeking" curve; where the rate of utility increases rapidly as the desired end of the scale (6%) is approached. Utility can also be defined by risk averse and linear curves.

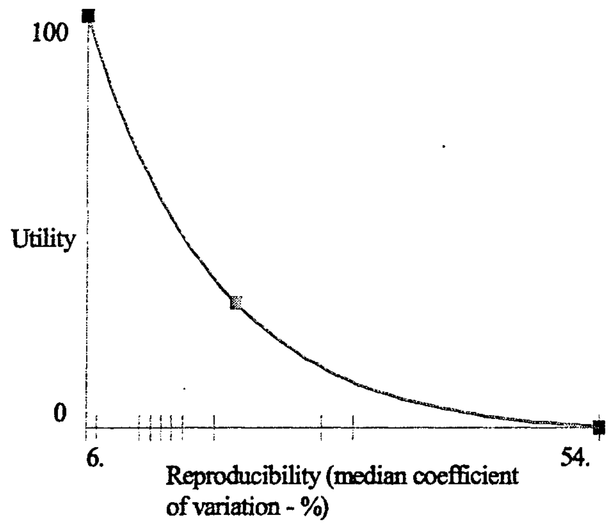


Figure 6: *Test Reproducibility* Measure Utility Curve Example

The final model development step was to weight the measures, based on their importance relative to the other measures. Measure weighting considers both relative priority and the concept of swing weighting. Swing weighting compares the effects of movement from the lowest point on the performance scale to the highest for any measure in relation to a similar move for any other measure. An example would be determining whether it was more important to move from "6%" to "54%" median coefficient of variation for the *Test Reproducibility* measure as compared to moving from "easy" to "demanding" for the *Storage Conditions Required* measure.

Several weighting techniques (Smarter Method, Analytical Hierarchy Process [AHP] and Direct Entry Assessment) were utilized to facilitate the development of the assessment model weights. The Smarter Method and the AHP weighting techniques were used as a starting point to establish measures weights, after which the measures weights were adjusted as necessary with the Direct Assessment technique, as this technique allows for a simple direct entry of weights.

The DAT developed a draft assessment model for the Fixed Base scenario based on the already defined technical requirements (see Appendix B). This model was reviewed and modified by the user representatives with input from the technical experts. The user representatives then created an assessment model for the Field/Contingency and Small Unit Scenarios. The Fixed Base Scenario model is presented in *Figure 7* (see Appendix I for Small Unit and Field/Contingency Scenario models and for a description of model components for all three models).

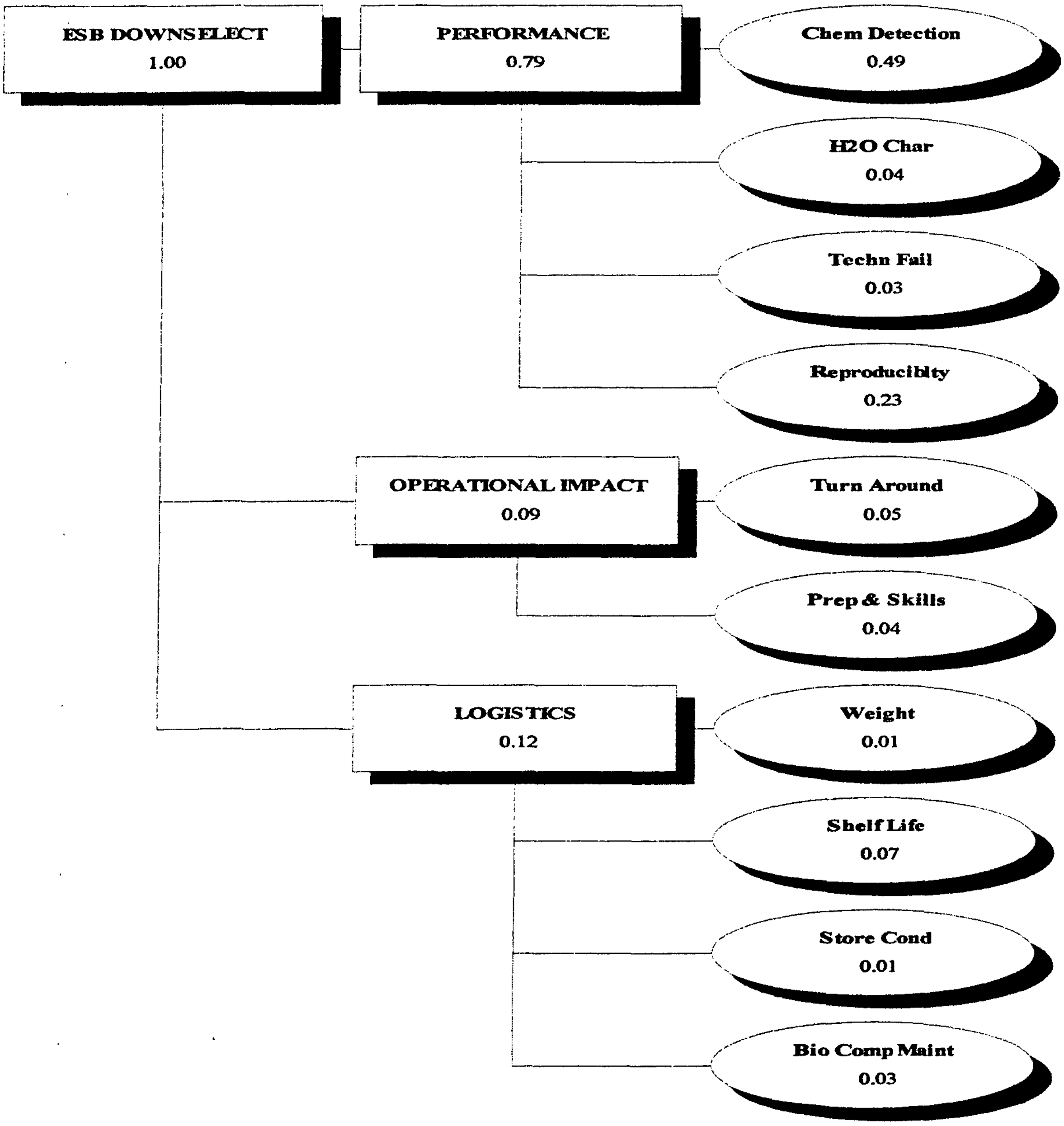


Figure 7: Fixed Base Scenario Model

Figure 8 shows the definitions for all ten measures, which are the same for the three models. *Figure 9* lists the performance scales plus weights for the three models and shows five of the ten measures differ in performance scales or utility functions. For example, for the *Biological Component Maintenance* measure, a technology requiring "some" biological component maintenance would score zero utility in the Small Unit Scenario model but a 60% utility in the Fixed Base scenario model. Nine of the ten measure weights are also different for each model. For example, the *Test Reproducibility* measure is weighted 23% in the Fixed Base Scenario model, 13% in the Field/Contingency Scenario model, and 9% in the Small Unit Scenario model (see *Figure 9* below). Only the *Chemical Detection* measure has the same weight in all models. Note this measure is worth almost 50% of each model's overall weight.

Measure Definitions for each Scenario/Model	
Measure	**Definition**
Chemical Detection	The ability of the technology to provide an adverse response to a set of representative chemicals at concentrations between the short-term MEG and the HLC for the greatest number of test chemicals. For this measure, it does not matter how close the technology provides an adverse response to the short-term MEG.
Water Characteristics	The ability to operate under a number of water quality conditions and in the presence of interfering substances with minimal effect on test outcome. The ability to operate in water containing residual chlorine is a surrogate for this measure.
Technology Failure Rate	The number of times a technology fails to produce viable/usable results per a certain number of tests. Excluded from this measure are failures caused by human mistake. For each technology evaluated the technology failure rate includes the biological basis of the system and all ancillary equipment.
Test Reproducibility	The ability of the ESB technology to produce the same output/answer given the same input and test conditions over multiple tests. This is measured as a median coefficient of variation; a low coefficient of variation represents high reproducibility.
Test Turn Around Time	The time required between consecutive tests (assumes reusable technology). Includes operator set-up time, sample preparation, technology operation time, and any time required for the system to reset before another reading.
Sample Preparation & Skills Required	The number of steps (few vs. many) and complexity of steps (e.g., measuring volume, adding reagent) to perform tests along with level if skills and facilities required (at sample site vs. in a lab) to complete tests. The focus is not on how long it takes to perform each task but rather how complicated each task is and what level of skills are required to complete the tasks.
Weight	The weight of the technology and necessary peripheral equipment (e.g., maintenance and repair supplies and parts, power source, and a 2-week supply of consumables).
Shelf Life	Length of storage life for the technology and consumables (i.e., how long will technology be usable [includes shipping time] under optimal conditions for that technology).
Storage Conditions Required	The conditions required to maintain the technology in operating condition for the maximum possible shelf life.
Biological Component Maintenance	Time required, complexity of system, and materiel and logistics required to maintain biological component of the test system.

Figure 8: Definitions of Measures for all Models

Measure Performance Scales and Weights for each Scenario/Model						
	Scenarios/Models					
	Fixed Base		Field Contingency		Small Unit	
Measure	Performance Scale	Wgt	Performance Scale	Wgt	Performance Scale	Wgt
Chemical Detection	100 = 6 chemicals 0 = 0 chemicals Linear continuous curve	49	100 = 6 chemicals 0 = 0 chemicals Linear continuous curve	49	100 = 6 chemicals 0 = 0 chemicals Linear continuous curve	49
Water Characteristics	100 = >5mg/L 75 = >2-5mg/L 50 = 1-2mg/L 0 = <1mg/L Discrete levels	4	100 = >5mg/L 75 = >2-5mg/L 50 = 1-2mg/L 0 = <1mg/L Discrete levels	2	100 = >5mg/L 75 = >2-5mg/L 50 = 1-2mg/L 0 = <1mg/L Discrete levels	1
Technology Failure Rate	100 = 0% failures 67 = >0 - 5% 33 = >5 - 15% 0 = >15% Discrete levels	3	100 = 0% failures 80 = >0 - 5% 30 = >5 - 15% 0 = >15% Discrete levels	6	100 = 0% failures 85 = >0 - 5% 20 = >5 - 15% 0 = >15% Discrete levels	5
Test Reproducibility	100 = 6% median coefficient of variation 0 = 54% Nonlinear continuous curve (same for all scenarios)	23	100 = 6% median coefficient of variation 0 = 54% Nonlinear continuous curve (same for all scenarios)	13	100 = 6% median coefficient of variation 0 = 54% Nonlinear continuous curve (same for all scenarios)	9
Test Turn Around Time	100 = 5 minutes 0 = 180 minutes Nonlinear continuous curve (same for all scenarios)	5	100 = 5 minutes 0 = 180 minutes Nonlinear continuous curve (same for all scenarios)	10	100 = 5 minutes 0 = 180 minutes Nonlinear continuous curve (same for all scenarios)	9
Sample Preparation & Skills Required	100 = few, minimal 67 = some, moderate 33 = many, significant 0 = many, expert Linear continuous curve	4	100 = few, no skills 90 = few, minimal 50 = some, moderate 0 = many, significant Linear continuous curve	6	100 = few, no skills 70 = few, minimal 0 = some, moderate Linear continuous curve	8
Weight	100 = 1lb 0 = 40lb S-shaped continuous curve (different for each scenario)	1	100 = 1lb 0 = 40lb S-shaped continuous curve (different for each scenario)	2	100 = 1lb 0 = 40lb S-shaped continuous curve (different for each scenario)	3
Shelf Life	100= >12 mo 0=< 0.5 mo Nonlinear continuous curve	7	100= >12 mo 75 = 6-12 mo 40 = 3-6 mo 0=< 3 mo Discrete levels	6	100= >12 mo 75 = 6-12 mo 30 = 3-6 mo 0=< 3 mo Discrete levels	6
Storage Conditions Required	100=Easy 50=Average 30=Demanding 0=Extremely Demanding Discrete levels	1	100=Easy 50=Average 0=Demanding Discrete levels	1	100=Easy 0=Controlled Discrete levels	4
Biological Component Maintenance	100 = None 60 = Some 0 = Media exchange or CO2 required Discrete levels	3	100 = None 40 = Some 0 = Media exchange or CO2 required Discrete levels	5	100 = None 0 = Some Discrete levels	6

Figure 9: Measure Performance Scales and Weights for all Models

Programmatic factors/measures such as cost, risk, and potential for the ESB technology's capabilities to improve are not included in the models. These decision factors, though, were considered along with the model's results in the final conclusions and recommendations. Technical experts provided important insights and data on these factors for the most promising ESB technologies (see Appendix J for programmatic information for top-ranked and recommended technologies). *Figure 10* is an example ESB technology programmatic assessment. Programmatic input/comments are very general because of the limited programmatic data about each technology (most technologies are in an early stage of development) and because of the limited time the technical experts had to complete this assessment.

Programmatics Assessment			
ESB Technology	**Cost (see Note 1, 2, & 3)**	**Risk (see Note 1 & 3))**	**Potential for Technology's Capabilities to Improve (see Note 1 &3)**
Microtox	Fairly reasonable for capital investment and maintenance. Some increased costs due to need to maintain cultures.	Low risk. Very mature technology.	Little potential for further improvement.
Note 1: Because technologies are in an early stage of development, because of the limited programmatic data about each technology, and because of the limited time the technical experts had to complete this assessment the programmatic input/comments are very general. Note 2: Costs don't include initial technology development costs but rather only costs to initially buy final technology product and maintain it to include supplies to operate it. Note 3: Maturity level of technology is considered in evaluation.			

Figure 10: Example of Technology Programmatics Assessment

2.6 Analysis of Results.

The DAT facilitated a two-day meeting in May 2005 where the technical experts completed the quantitative downselect assessment. Technical experts used a combination of both quantitative and qualitative data about the ESB technologies along with their knowledge of similar technologies and mechanisms. Final scores were a consensus of expert judgment, not an average of the individual technical expert assessments. Assumptions and rationales for scores were documented where necessary.

Overall scores were calculated for each technology by multiplying the numerical rating from the performance scale by the weight of each measure, and summing the scores over all measures (linear additive function). The highest possible score would be 100. While the overall scores are important, they are only used as a guide to formulate findings and conclusions.

The analyses of results were completed by the DAT from three different perspectives. First, the overall results were examined to discern general outcomes and trends. Second, each technology was examined to identify strengths and weaknesses. Third, the results specific to each measure were examined to identify potential technical obstacles or shortcomings. This examination included performing sensitivity analysis to determine what effect varied measure weights would have on the results. LDW was used in the analysis because it has many useful tools for performing comparative analysis and displaying and documenting results.

2.6.1 Overall Results.

Figures 11-16 show the overall score and rank of each ESB technology for the three models. The figures also show where each technology is strong and weak, relative to the three goals and ten measures of the model. The first figure for each scenario (see *Figures 11, 13, 15*) presents the overall results by showing the results of how each technology performed against the three goals. The second figure for each scenario (see *Figures 12, 14, 16*) shows similar information as what the first figure presents, but presents information in a greater level of detail by showing how each technology scored against the ten measures. The length of each of the sub-bars indicates how much of the technology's overall score is attributable to the three goals or ten measures (note the pattern of a bar in the stack bar chart may change from model to model [e.g., the *Shelf Life* measure bar pattern is different in *Figures 12 and 14*]).

2.6.1.1 Fixed Base Scenario.

In the Fixed Base Scenario model (*Figures 11-12* show results) the ESB technologies can be categorized into five broad ranking tiers: significantly above average (Microtox), above average (Hepatocyte Low Density Lipoprotein Uptake, Electric Cell-Substrate Impedance Sensing, Mitoscan Electron Transfer), average (Eclox, Neuronal Microelectrode Array, SOS Cytosensor, Toxi-Chromotest), below average (ToxScreen Organic, ToxScreen Metal, *Sinorhizobium meliloti* Assay), and significantly below average (Mitoscan Reverse Electron Transfer).

Figure 11 illustrates the overall technology scores and ranking relative to the three model goals. This figure indicates that the Performance goal is the strongest discriminator, primarily due to its high weight (79%; refer to *Figure 9* for the list of weights for each measure in each model), but also due to the differing scores among most of the ESB technologies. The Logistics goal has a lesser effect, mainly because of its lower weight in the model (12%). The Logistics goal provides the most differentiation among the top three technologies as they score similarly against the Performance goal. The Operational Impact was the weakest discriminator partly because this was the least weighted goal (9%).

Figure 12 illustrates the overall technology scores and ranking relative to the ten model measures. This figure provides a greater level of detail for each technology because it provides data for each of the ten measures used in the model. For example, the figure shows that the SOS Cytosensor received about 80% of its overall score from one measure, *Chemical Detection*, while Neuronal Microelectrode Array scored almost 95% of its overall score from two measures (*Chemical Detection* and *Test Reproducibility*). This figure also shows, for example, *Chemical Detection* and *Test Reproducibility* measure, the two highest weighted measures, did not provide much discrimination between Microtox, Hepatocyte Low Density Lipoprotein Uptake, and Electric Cell-Substrate Impedance Sensing.

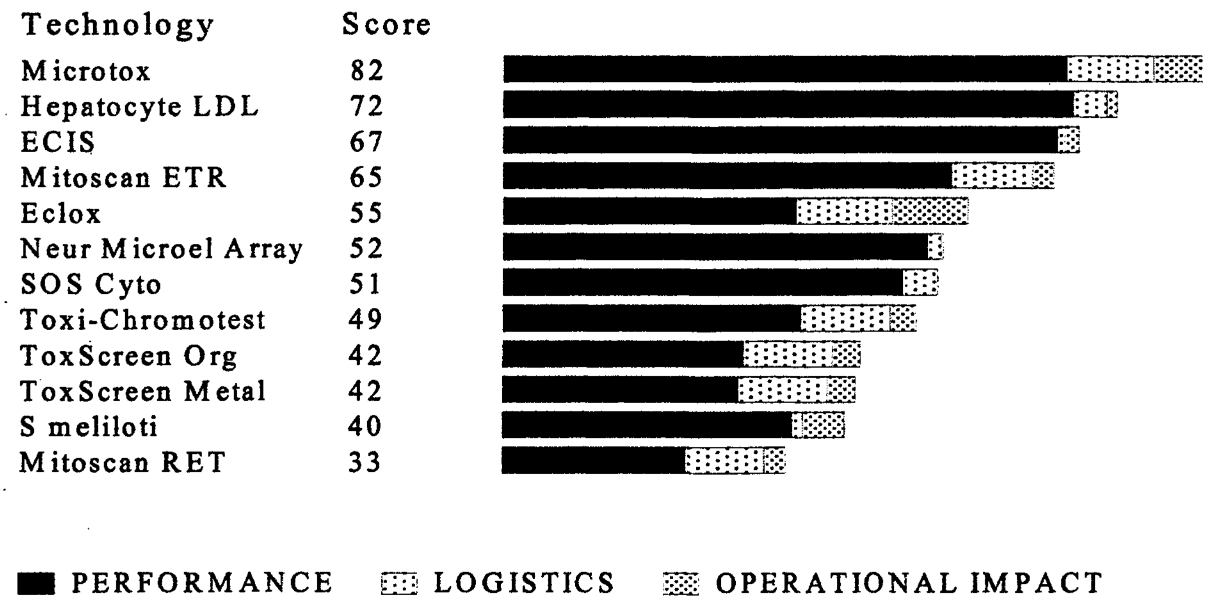


Figure 11: Overall Assessment Goal Scores for ESB Technologies: Fixed Base Scenario

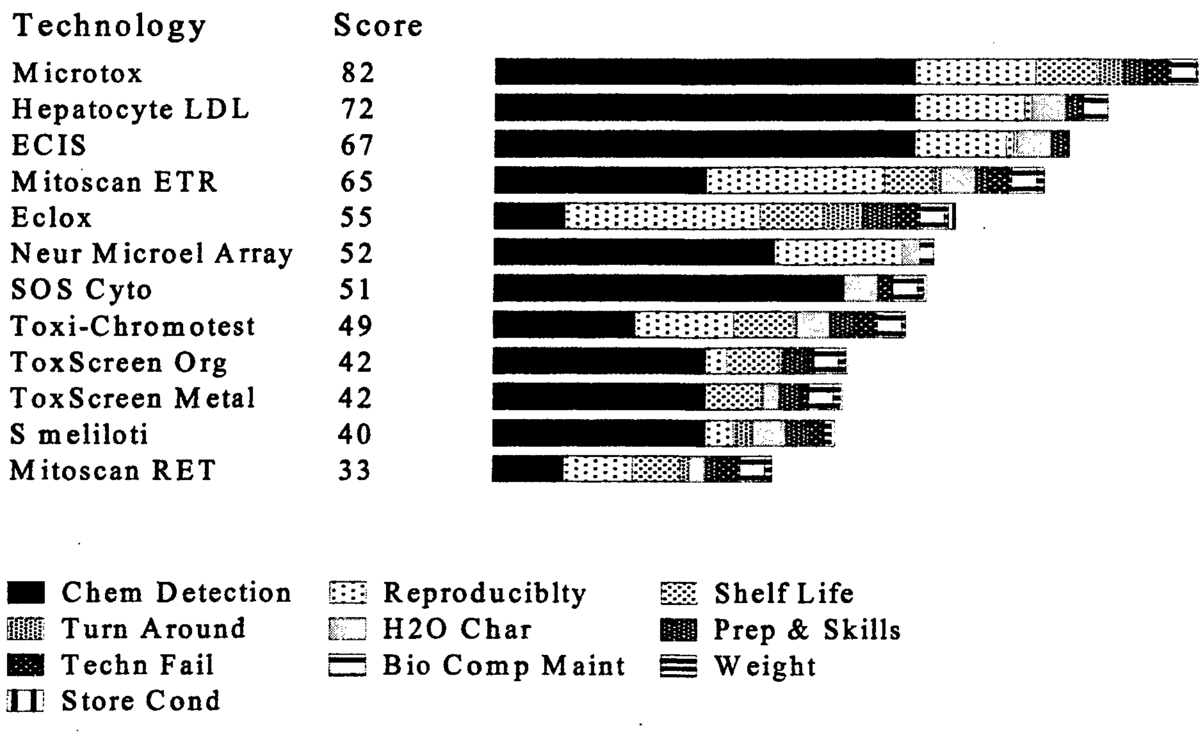


Figure 12: Overall Assessment Measures Scores for ESB Technologies: Fixed Base Scenario

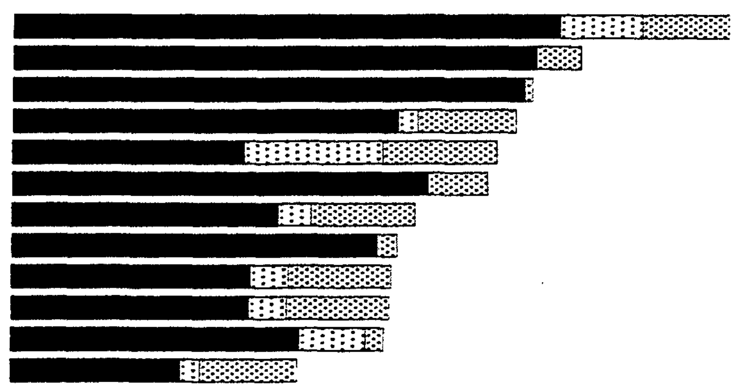
2.6.1.2 Field/Contingency Scenario.

In the Field/Contingency Scenario model (*Figures 13-14* show results) the ESB technologies can be categorized into the same five broad ranking tiers as the Fixed Base Scenario model: significantly above average (Microtox), above average (Hepatocyte Low Density Lipoprotein Uptake), average (Electric Cell-Substrate Impedance Sensing, Mitoscan Electron Transfer, Eclox, SOS Cytosensor), below average (Toxi-Chromotest, Neuronal Microelectrode Array, ToxScreen Organic, ToxScreen Metal, *Sinorhizobium meliloti* Assay), and significantly below average (Mitoscan Reverse Electron Transfer).

In this scenario, the Operational Impact and Logistics goals are more important (31% of model's weight; refer to *Figure 9* for the list of weights for each measure in each model) than in the Fixed Base Scenario (21%). Most ESB technologies dropped in score, because of the relatively higher Operational Impact and Logistics goal weights, versus how they scored in the Fixed Base Scenario model. The exceptions are the top scoring technology (Microtox) and the lowest scoring technology (Mitoscan Reverse Electron Transfer), which scored the same, and Eclox and SOS Cytosensor, which have slightly higher scores.

The technologies scored overall in the same ranking tiers as in the Fixed Base Scenario model except for Mitoscan Electron Transfer which dropped from the above average tier to the average tier and Toxi-Chromotest and Neuronal Microelectrode Array which dropped from the average tier to the below average tier. Microtox is still the best overall performer.

Technology	Score
Microtox	82
Hepatocyte LDL	65
ECIS	60
Mitoscan ETR	58
Eclox	56
SOS Cyto	55
Toxi-Chromotest	46
Neur Microel Array	44
ToxScreen Org	44
ToxScreen Metal	44
S melitoti	43
MitoscanRET	33



PERFORMANCE OPERATIONAL IMPACT LOGISTICS

Figure 13: Overall Assessment Goal Scores for ESB Technologies: Field/Contingency Scenario

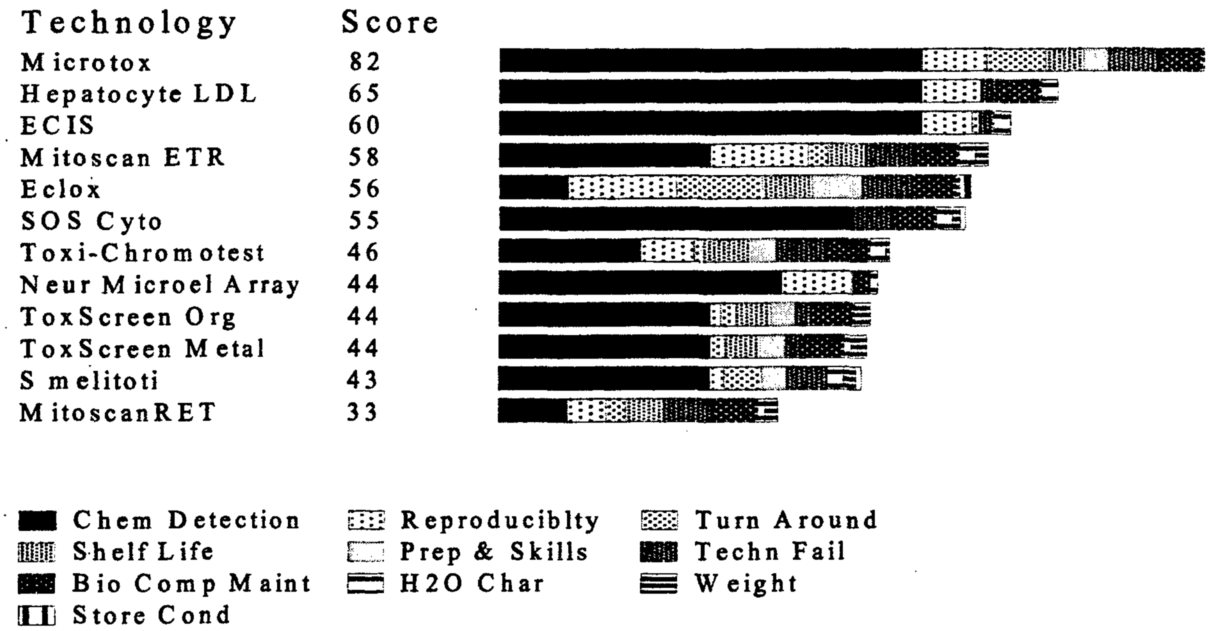


Figure 14: Overall Assessment Measures Scores for ESB Technologies: Field/Contingency Scenario

2.6.1.3 Small Unit Scenario.

In the Small Unit Scenario model (*Figures 15-16* show results) the ESB technologies can be categorized into the same five broad ranking tiers as the other models: significantly above average (Microtox), above average (Hepatocyte Low Density Lipoprotein Uptake), average (Electric Cell-Substrate Impedance Sensing, Eclox, Mitoscan Electron Transfer, SOS Cytosensor), below average (Toxi-Chromotest, Neuronal Microelectrode Array, ToxScreen Organic, ToxScreen Metal, *Sinorhizobium meliloti* Assay), and significantly below average (Mitoscan Reverse Electron Transfer).

In this scenario, the Operational Impact and Logistics goals are even more important (31% of model's weight; refer to *Figure 9* for the list of weights for each measure in each model) than in the Field/Contingency (31%) and the Fixed Base (21%) Scenario models. All ESB technologies stayed in the same ranking tier as the Field/Contingency Scenario model., although all technology's scores dropped. The drop in score is because of the relatively higher Operational Impact and Logistics goals, versus how they scored in the Field/Contingency or Fixed Base Scenario models. Microtox is still the highest scoring technology by a wide margin, but fell the most in overall score. Microtox's scored dropped from 82 to 76. *Figure 15* shows that Mitoscan earns about the same overall score with the *Performance* goal as Eclox earns with two goals (*Performance and Logistics*).

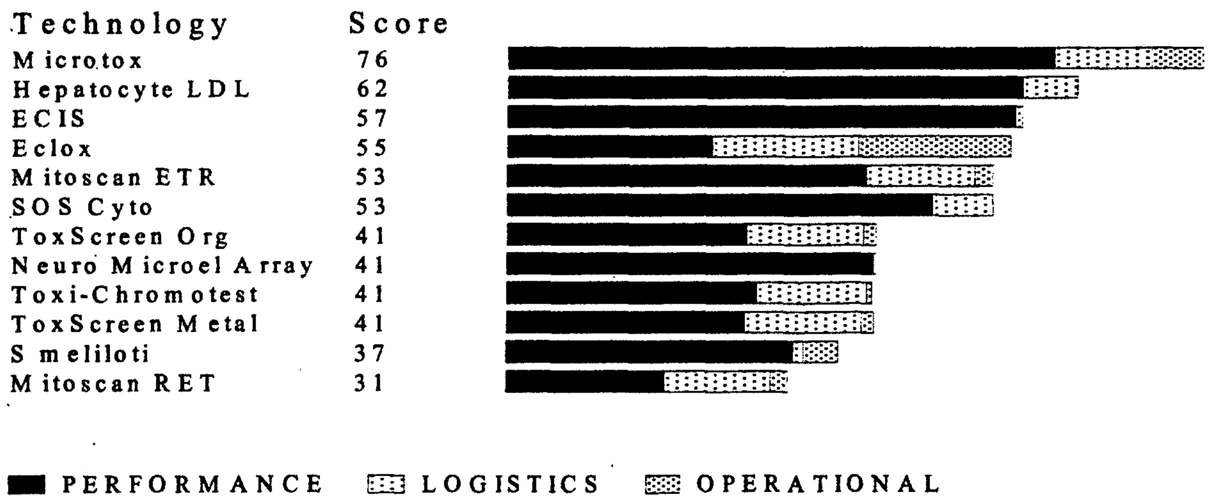


Figure 15: Overall Assessment Goal Scores for ESB Technologies: Small Unit Scenario

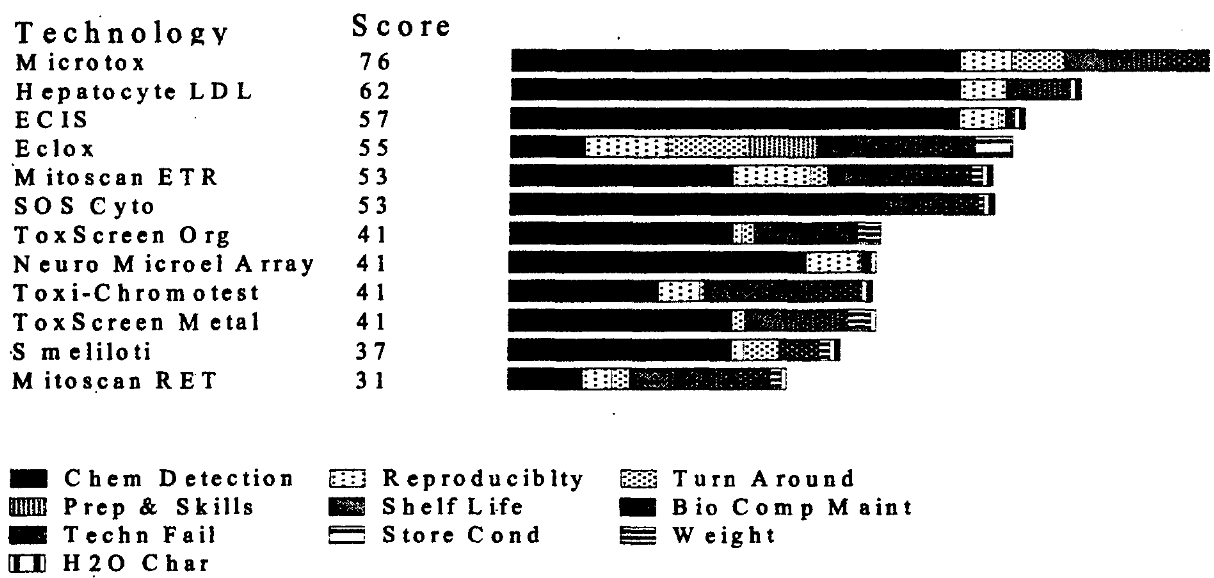


Figure 16: Overall Assessment Measures Scores for ESB Technologies: Small Unit Scenario

2.6.2 Technology Analysis.

The purpose of the technology analysis is to highlight areas where particular technologies stand out, either positively or negatively. Appendix K contains a narrative summary of the relative strengths and weaknesses for each technology, as well as charts that show how each technology performed relative to the ten measures. *Figure 17* is an example of a narrative summary. For the narrative summaries, "above average and below average" descriptors are used. These were subjectively determined by the DAT and are based on each ESB technology's score relative to the other ESB technology score.

Microtox (82) scored above average on six measures (*Chemical Detection, Technology Failure Rate, Test Turn Around Time, Preparation and Skills Required, Shelf Life, and Biological Component Maintenance*) and below average on *Water Characteristics, Weight,* and *Storage Conditions Required* measures. Microtox detected six chemicals, including one chemical the other three top-ranked technologies did not detect - cyanide.

Figure 17: Example of a Technology Strengths and Weaknesses Analysis Narrative Summary

LDW was used to generate each technology's strengths and weaknesses analysis chart. The charts graphically show a particular technology's strengths and weaknesses, relative to each measures weights. *Figure 18* is an example of a technology's strengths and weaknesses analysis chart. The height of the bars indicates a technology's relative score for each measure, while the width indicates the relative weight of the measure. This chart shows that Microtox scores very high against four measures, *Chemical Detection* (highest weighted measure also), *Shelf Life, Biological Component Maintenance,* and *Technology Failure Rate.* The measures where Microtox scored low were generally weighted low, for example the *Water Characteristics* measure (H2O Char, 6th bar from the left).

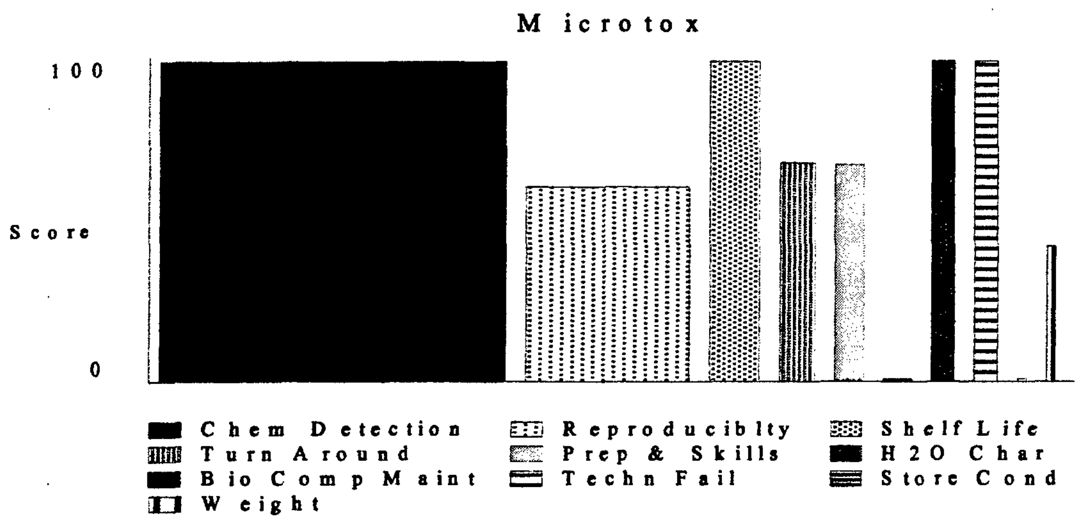


Figure 18: Example of a Technology Strengths and Weaknesses Analysis Chart

2.6.3 <u>Measure Analysis</u>.

The measures analysis summarizes the assessment results in terms of individual measures (*Figure 19* is an example). Because the measures represent user needs, this analysis helps identify areas of shortfall, or potential technical challenges (e.g., if the ESB technologies generally score low against a measure). Conversely, the analysis also identifies areas of minimal concern (e.g., the technologies generally score high and/or the measure is low-weighted).

The range of scores for each measure was examined to determine overall ESB technology performance relative to each measure, which provided the basis for assignment of a

subjective assessment rating by the DAT. Comments and rationale for each measure rating were also provided. Each measure was assessed on a green, yellow, red scale (see *Figure 19* for definition of rating). All measures were rated either green or yellow – no measure was rated red. Reference Appendix L for the assessment/rating and Appendix M for the sensitivity charts for all measures.

Measures (Wgt)	Rating (see Rating Key)	Comments
Chemical Detection (0.49)	Green (see Note 1)	- This measure is weighted the highest, almost half of the model's weight. - The utility function is a linear continuous curve. For each chemical detected the technology earns 16.7% of the possible score. For example, detecting four chemicals would earn a score of 66.8 (4 x 16.7) out of 100. - Seven technologies detected three chemicals or fewer and thus earned half or less of the possible maximum score. Note 1: Because the maximum number of chemicals detected by any technology is six, this is the 100 score, but it is a goal to detect all twelve chemicals. In order to detect more than six chemicals, an ESB technology "system" is needed (i.e., two or more toxicity sensors working together).
Rating Key: Green: Area of low concern indicating user needs should be met, or the measure is low weight and is unlikely to cause significant impact. Yellow: Area of moderate concern indicating user needs may not be met. Red: Area of high concern indicating user needs are not readily met.		

Figure 19: Example of a Measure Analysis

Sensitivity analysis was also performed to determine whether changes in measures weights would affect the results. This analysis was conducted on all measures. LDW was used to generate sensitivity graphs for each measure. *Figure 20* shows a typical example of a technology's sensitivity analysis chart. The sensitivity graphs contain the overall score on the y-axis (from worst to best) and the percent of the weight given to the measure being evaluated on the x-axis (from 0 to 100). There is a line segment for each ESB technology on the graph, which extends from the x-axis at various angles to the position that corresponds with 100% of the weight being assigned to that measure. A vertical line on the sensitivity graph identifies the current weight assigned to the measure. The order in which the vertical weight line intersects the alternatives' segments is the overall rank order produced by the model's weighting scheme.

By visually noting the changes in rank order which would occur if the vertical weight line was moved to the right or left (measure weight increased or decreased), an assessment can be made as to the sensitivity of the weight of that measure. If a slight movement of the weight line causes multiple alternative lines to cross (and therefore the rank order to have multiple changes), then that measure would be considered very sensitive to weight changes. If, on the other hand, no ranking changes occur when the line is moved a greater distance, then it

can be determined that the weight of that measure does not have a notable effect on the outcomes of the analysis.

For the most part, the technology rankings are insensitive to reasonable (± 15% changes) measure weight changes. This is especially true for the top three scoring technologies (Microtox, Hepatocyte Low Density Lipoprotein Uptake, and Electric Cell-Substrate Impedance Sensing). There are two instances were reasonable weight changes have an impact:

- The first instance is when the *Chemical Detection* measure weight is increased or reduced 15% (considered maximum reasonable change; see *Figure 20*). By decreasing the weight of the *Chemical Detection* measure 15% Mitoscan Electron Transfer and Eclox change positions in the ranking (3rd and 4th positions). Toxi-Chromotest increases from 8th highest ranked to 6th highest ranked. By increasing the weight of the *Chemical Detection* measure 15%, the impact on the ranking is a little less as only SOS Cytosensor increases from 7th highest ranked to 5th highest ranked and exchanges ranking with Eclox.
- The second instance is when the weight of the next highest weighted measure, *Test Reproducibility*, decreases 15% (See Appendix M, *Figure M-4*). When this happens, SOS Cytosensor increases from seventh highest ranked to fifth highest ranked. Eclox and Neuronal Microelectrode Array each drop one position in ranking. When the measure is increased 15% there is only a slight impact - Electric Cell-Substrate Impedance Sensing and Mitoscan Electron Transfer would rank the same and SOS Cytosensor and Toxi-Chromotest would rank the same.

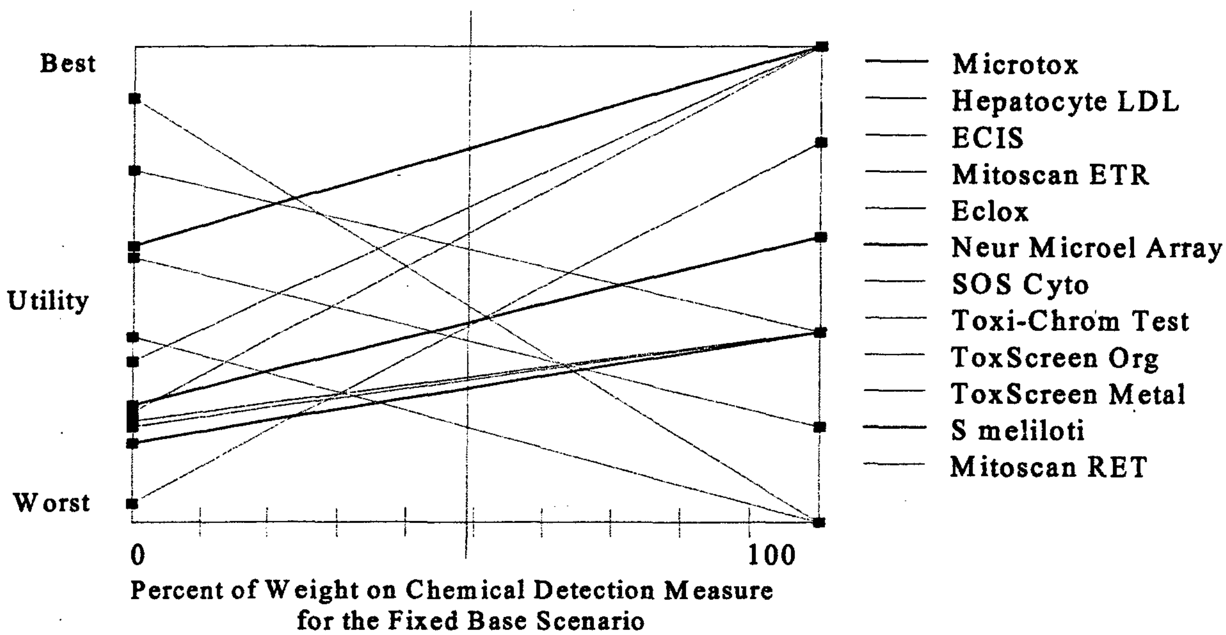


Figure 20: Example of a Measure Sensitivity Analysis Chart

3. CONCLUSIONS

No technology detected more than half of the chemicals required within the MEG-HLC range. All chemicals except for nicotine were detected by at least one technology. Three chemicals were only detected by one technology. Only Neuronal Microelectrode Array detects aldicarb and methamidophos and only Electric Cell-Substrate Impedance Sensing detects ammonia.

By creating a system of three ESB technologies it would be possible to detect nine out of the twelve chemicals. It would take five technologies to detect all twelve chemicals.

Even the best three ESB technologies only scored in the 67-82 range with the Fixed Base Scenario model and thus have areas within performance, operational impact, or logistics that could be improved.

ESB technologies that scored poorly in the models but can detect an important class of chemicals may be recommended for further considered and research. For example, the Daphnia IQ Test, a technology eliminated from consideration with the qualitative screening model, is the only technology known to detect nicotine at the appropriate sensitivity level.

4. RECOMMENDATIONS

Expand the current chemical training/test set beyond twelve to include other classes of chemicals and additional chemical examples for the current classes of chemicals studied. These additional chemicals are needed to enhance the project team's understanding and confidence levels of toxicity responses for the ESB technologies chosen for further study and development.

Adopt an evolutionary development strategy for an ESB system. Initially focus on the Fixed Base Grab Sample Scenario because this provides the greatest chance of program success (i.e., fielding a prototype before FY09). Add other use scenarios in future program increments, as ESB technologies evolve and improve.

Recommend Microtox (or Deltatox), Hepatocyte Low Density Lipoprotein Uptake Uptake, Electric Cell-Substrate Impedance Sensing, Neuronal Microelectrode Array, and Daphnia IQ be further researched and developed. This recommendation is based on the fact that Microtox (or Deltatox, which is a field portable version of Microtox), Hepatocyte Low Density Lipoprotein Uptake Uptake, and Electric Cell-Substrate Impedance Sensing together detect nine different chemicals and scored the highest in the Fixed Base Scenario model. The recommendation for Neuronal Microelectrode Array and Daphnia IQ Test is because they detect three chemicals that Microtox, Hepatocyte Low Density Lipoprotein Uptake, and Electric Cell-Substrate Impedance Sensing do not. These five technologies detect all twelve chemicals. Note that Neuronal Micoelectrode Array and Daphnia IQ Test scored below average in the models as they both have logistical and operational issues that must be addressed.

Use the results from each ESB technology's measure assessment (see Appendix L) to help identify areas to focus research and development (e.g., increase Microtox's ability to handle varying water characteristics).

Eclox is not recommended for further assessment or consideration. This is because it only detects one chemical (copper). The top four highest scoring ESB technologies also detect copper and Eclox detects copper much closer to the HLC than the short-term MEG. Eclox rated very high, though, on almost all measures in the model except for *Chemical Detection*, *Water Characteristics*, and the *Weight* measures. The technical experts especially liked the field portability and maturity level of Eclox.

Although the initial focus of the ESB ATO may be to deliver an ESB prototype system suitable for the Fixed Base Scenario, it is important to strive to achieve the requirements for all six scenarios. Seed money should be considered for some ESB technologies that may not be ready for near-term fielding but that may be the best option long term for scenarios other than the Fixed Base Scenario.

Examples of technologies that the technical experts have determined don't have a reasonable potential to meet the current ATO requirements but that could someday possibly meet the threshold requirements for the other five scenarios in the out years are SOS Cytosensor, Stressor-Specific *Escherichia coli* Sensor, Cell Culture Analog, Multianaltye Micro- and Nano-Physio-meters, *Caenorhabditis elegans* Monitor, and Instant Multicellular Organisms. These technologies should be put on a technology watch list. The technical experts also recommended it be determined if a well-developed version of SOS Cytosensor already exists.

Resource allocation decision support methodologies could be used to model the various funding options for each of the technologies chosen. This would allow the ESB system program management to perform "what-if" analysis and model how to maximize the benefits while addressing potential funding cuts or additions.

Decision analysis methodologies and tools can provide a framework to further analyze current and new ESB technologies as additional data from future research becomes available, perhaps as part of a structured reassessment process.

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APPENDIX A

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM DOWNSELECTION PROJECT TEAM		
First name	**Last Name**	**Affiliation**
Ric	De Leon	Metropolitan Water District of Southern California
Jay	Dusenbury	US Army Research, Development and Engineering Command
Tom	Gargan	U.S. Army Center for Environmental Health Research
Mike	Goode	Edgewood Chemical Biological Center
John	Harbell	Institute for In Vitro Sciences
Wally	Hayes	Harvard School of Public Health
Janet	Jensen	Edgewood Chemical Biological Center
Randy	Kellar	Boeing, Inc. (Representative for Lead System Integrator, Future Combat Systems – Manned Ground Vehicle program)
Scott	Kooistra	Edgewood Chemical Biological Center
Joe	Pancrazio	National Institutes of Health (Neurological Disorders and Stroke)
Steve	Richards	U.S. Army Center for Health Promotion and Preventive Medicine
Robert	Ryczak	U.S. Army Center for Health Promotion and Preventive Medicine
Stanley	States	Pittsburgh Water & Sewer Authority
Roy	Thompson	Edgewood Chemical Biological Center
Bill	Van der Schalie	U.S. Army Center for Environmental Health Research
Jeremy	Walker	US Army Research, Development and Engineering Command
John	Walther	Edgewood Chemical Biological Center
Lindsey	Wurster	Edgewood Chemical Biological Center

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APPENDIX B

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
1	Detection	Does device need to determine a fixed level of toxicity only (e.g., acute or MEG) or give an adjustable/sliding scale reading (e.g., giving a reading between acute and MEG)?	Detect and trigger at a fixed level (Acute[T]).	Detect and trigger at a fixed level (Acute[T]).	Detect and trigger at a fixed level (Acute, MEG-listed TICs for short term consumption [T]). Detect and trigger at two fixed level (Acute & MEG-listed TICs at 1-yr consumption levels [O]).	Detect and trigger at a fixed level (Acute [T]). Detect and trigger at two fixed level (Acute & MEG [O]).	Detect and trigger at a fixed level (Acute [T]). Detect and trigger at three fixed level (Acute, MEG, & EPA [O]) but even better is a sliding scale reading for Acute, MEG, and EPA (O).	Detect and trigger at a fixed level (Acute [T]). Detect and trigger at three fixed level (Acute, MEG, & EPA [O]) but even better is a sliding scale reading for Acute, MEG, and EPA (O).
2	Detection	What toxic industrial chemicals need to be detected? Chemicals that are most important to detect? For example, which are more important the chemicals that effect cognitive functions or gastrointestinal (GI) functions?	CNS/CVS effects w/r to short-term MEG-listed TICs (T) Above + PNS, liver, kidneys (O1), Above + GI tract (O2)	CNS/CVS effects w/r to short-term MEG-listed TICs (T) Above + PNS, liver, kidneys (O1), Above + GI tract (O2)	MEG-listed TICs with CNS/CVS effects (T) Above + PNS, liver, kidneys, GI tract (O)	MEG-listed TICs with CNS/CVS effects (T) Above + PNS, liver, kidneys, GI tract (O)	TICs with CNS/CVS effects (T) Above + PNS, liver, kidney, GI tract (O)	TICs with CNS/CVS effects (T) Above + PNS, liver, kidney, GI tract (O)

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
3	Detection	What's the level of detection for each chemical?	Acute, i.e. MEG-short term (T)	Acute, i.e. MEG-short term (T)	MEG - short + long term (O)-Acute, i.e., MEG -short term(T)	MEG - short + long term (O)-Acute, i.e., MEG -short term(T)	EPA(O)-MEG (O)-Acute (T)	EPA(O)-MEG (O)-Acute (T)
4	Environmental Conditions During Test	Range for air temperature, atmospheric pressure/altitude, and humidity level?	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)
5	Environmental Conditions During Test	Other water characteristics device must handle (e.g., quantity of total dissolved solids, turbidity, chlorine residual)?	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
6	Environmental Conditions During Test	Range for water pH and temperature?	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)
7	Logistics	Power requirements? Amount? Type (e.g., solar, AC[110, 220], DC [battery])?	Internal power source (T)	External power (T), Internal power source (O)	External power (T), Internal and external power source (O)	External power (T), Internal and external power source (O)	External power (T), Internal and external power source (O)	External power (T), Internal and external power source (O)
8	Logistics	Size and weight of support requirements (consumables, replacement parts, storage)	7 days worth of consumables (15 liters/day per person * 1.5) (T) 14 days (O)	7 days worth of consumables (15 liters/day per person * 1.5) (T) 14 days (O)	1 cu ft/5 lbs (T) 1/2 cu ft/2.5 lbs (O)	2 cu ft/10 lbs (T) 1 cu ft/5 lbs (O)	Minimal concern for installation testing	Minimal concern
9	Physical Characteristics	Controls (dials, switch, knob, etc.) operable in protective and cold weather suits	MOPP (T)	MOPP (T)	MOPP (T)	MOPP (T)	Level A (T)	Level A (T)

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
10	Physical Characteristics	Display (visible in low light conditions, no sound, remote alarm for continuous monitoring)	Visible in low light conditions, no sound (T)	Visible in low light conditions, no sound (T)	Visible in low light conditions, no sound (O)	Visible in low light conditions, no sound (O). Remote alarm (O)	No requirement	Remote alarm (O)
11	Physical Characteristics	Max. cube, length, and weight?	Credit card size (O). (T) Needs to be researched further.	Max 1 cu ft, 10 lbs (O)	Two cubic feet, 20 lbs (T) One cubic foot, ten lbs (O)	Two cubic feet, 20 lbs (T) One cubic foot, ten lbs (O)	Field device (see Field/Contingency info) or installation level device (6 cu ft/ 40 lbs (T))	Could be as large as a portable lab trailer, as long as can tap pipe continuously (T). 6 cu ft/40 lbs (O)
12	Robustness	Does device still work if it becomes immersed in water or dropped (from 3 feet onto concrete)?	Yes (T)	Yes (T)	Yes (T)	Yes (T)	Yes (O)	Yes (O)
13	Robustness	If reusable, do or can TICs accumulate on device from test to test (to create a false positive)?	No (T)	No (T)	No (T)	No (T)	No (T)	No (T)

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
14	Robustness	Device has a indicator that ESB system is still working (or has a malfunction signal)?	Yes (T)	Yes (T)	Yes (T)	Yes (T)	Yes (T)	Yes (T)
15	Robustness	Number of tests (or hours if using continuous collection method) before reloading, recalibration?	One test (T), 150 tests (O)	One test (T), 150 tests (O)	One test (T), 150 tests (O)	20 hrs (T), 168 hrs (O)	One test (T), 150 tests (O)	24 hrs (T), 168 hrs (O)
16	Robustness	Reliability? Device failure rate per X amount of tests (or time if using continuous collection method)?	one per 850 tests (T & O)	one per 850 tests (T & O)	one per 850 tests (T & O)	280 hrs (20 hrs/day for 14 days) (T) 560 hrs (20 hrs/day for 28 days) (O)	one per 850 tests (T & O)	30 days (T), 60 days (O)
17	Robustness	Shelf life (device and consumables)?	1 yr (T), 2 yr (O)	1 yr (T), 2 yr (O)	1 yr (T), 2 yr (O)	1 yr (T), 2 yr (O)	30 days (T), 1 yr (O)	30 days (T), 1 yr (O)

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
18	Safety, Health, & Environmental	What are the requirements related to the safety, health of the user and the environmental impact?	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)
19	Testing Characteristics	Min. false positive readings and false negative readings (for specified chemicals). But which is more important (how much)?	false + <1/1000 (T), <1/5000 (O) false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O) false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O) false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O) false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O) false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O) false - <1/5000 (T), <1/10,000 O)
20	Testing Characteristics	Minimum time between consecutive tests (assumes reusable device)	30 min (T), Immediate (O)	Time to refill reservoir (T), Time to produce 2 liters of water (O)	1 hr (T), Immediate (O)	N/A	1 hr (T), Immediate (O)	N/A
21	Testing Characteristics	Sample prep complexity (e.g., measured volume, reagent addition)	None (T) None (O)	<5 steps (T), none . (O)	raw: 2 steps (T) none(O) Treated: 5 steps (T), none (O)	None after initial set up	<5 steps (T), none (O)	None after initial set up

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
22	Testing Characteristics	What is the test turn around time (time from starting test [i.e., includes set-up time] until knowing the results) ?	1 min (T) 15 sec (O)	2 min (T) 15 sec (O)	10 min (T) 2 min (O)	5 min (T) 1 min (O)	10 min (T) 5 min (O)	10 min (T) 5 min (O)
23	Testing Characteristics	Time to complete test (operator time to set up and sample)?	30 sec (T)5 sec (O)	1 min (T)30 sec (O)	5 min (T)30 sec (O)	None after initial set up	5 min (T)2 min (O)	None after initial set up
24	Testing Characteristics	Type of test results needed: Go-No Go vs. performing a procedure (e.g., look up/analysis)?	Simple (Go-No Go) (T)	Simple (Go-No Go) (T)	More Complicated than Go-No Go (T), Go -No Go (O)	More Complicated than Go-No Go (T), Go -No Go (O)	More complicated than Field/ Contingency Threshold (T), Go-No Go (O)	More complicated than Field/ Contingency Threshold (T), Go-No Go (O)
25	User Requirements	What is the level of skills and knowledge required to perform maintenance and calibration tests (field thus soldier/ simple or depot thus technician/ moderately complex)?	None (T) None (O)	None (T) None (O)	Medium (T) Low (O)	Medium (T) Low (O)	Moderate (T) Medium (O)	High (T) +Moderate (O)

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or ideal/preferred.

#	Assessment Category	Tech Requirements	Scenarios					
			Individual/Small Team	Small Unit	Field/Contingency		Fixed Base	
			Test Raw Water	Test Raw/Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
			Grab Sample Collection Method	Grab Sample Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method	Grab Sample Collection Method	Continuous Flow Collection Method
26	User Requirements	What is the level of skills and knowledge required to perform tests (soldier/simple vs. technician/moderately complex)?	Low (soldier simple) Dip, read, interpret result (go/no-go) (T,O)	Low (soldier simple) Fill sample vial, add reagent, dip, read, interpret result (go/no-go)	Medium (T) Low (O)	Medium (T) Low (O)	Moderate (T) Medium (O)	High (T) +Moderate (O)

APPENDIX C

ADDITIONAL INFORMATION ON MILITARY EXPOSURE GUIDELINES

Military Exposure Guidelines (MEGs) are provided for water (and other media) to estimate a level "… above which certain types of health effects may begin to occur in individuals amongst the exposed population". MEGs are "… designed to indicate 'thresholds' for minimal to no adverse health effects" and are considered "… protective against any significant non-cancer effects". MEG exposure scenarios for water are appropriate for a deployed military operation, i.e., exposure lasting either 5 or 14 days, with water consumption of either 5 or 15 L/day. The exposed population is defined to include "… relatively healthy and fit male and non-pregnant female adults", 18 to 55 years old with and average weight of 70 kg. It is important to note that while MEGs are not enforceable military standards, the MEGs are considered guideline concentrations for identifying and ranking Occupational and Environmental Health (OEH) risks. MEGs have been established for about 190 chemicals. MEGs provide a reference point above which adverse effects may be expected after a field-relevant period of exposure and may serve as lower thresholds for toxicity sensor responses. That is, responses at concentrations below the MEGs indicate toxic effects that may not be relevant to acute human health impairments.

Reference: U.S. Army Center for Health Promotion and Preventive Medicine. 2001. Chemical Exposure Guidelines for Deployed Military Personnel. TG-230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.

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APPENDIX D

CANDIDATE ENVIRONMENTAL SENTINEL BIOMONITOR

#	Technology Name	Biological System
1	AquaSentinel	Algae
2	Fluotox	Algae (Scendedesmus subspicatus)
3	Lumitox	Algae (Pyrocystis lunula)
4	Amtox	Bacteria
5	Baroxymeter	Bacteria
6	BioTox Flash Test	Bacteria (Vibrio fischeri)
7	Cellsense	Bacteria, algae
8	GreenScreen EM	Yeast (*Saccharomyces cerevisiae*)
9	LUMIStox	Bacteria (Vibrio fischeri)
10	MetPlate	Bacteria (Escherichia coli)
11	Microtox, Deltatox	Bacteria (Vibrio fischeri)
12	Polytox	Bacteria
13	*Sinorhizobium meliloti* Toxicity Test	Bacteria (*Sinorhizobium meliloti*)
14	STIPTOX	Bacteria
15	Stressor-Specific *E. coli* Sensor	Bacteria (*Escherichia coli*)
16	Toxi-Chromo Test	Bacteria (*Escherichia coli*; heavy metal sensitive variant)
17,18	ToxScreen (two types: Metal and Organic)	Bacteria (*Photobacterium leiognathi*)
19	ToxTrak	Bacteria
20	Vitotox	Bacteria (*Salmonella tyhimurium*)

CANDIDATE ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES		
#	**Technology Name**	**Biological System**
21	Aquanox	Enzyme (horseradish peroxidase)
22	Eclox	Enzyme (horseradish peroxidase)
23,24	Mitoscan (two types: Electronic Transfer [ETR] and Reverse Electron Transfer [RET])	Enzyme (sub-mitochondrial particles)
25	ArrayScan HCS System	Mammalian cells
26	Brain on a Chip	Mammalian cells
27	CANARY Biosensor	Mammalian cells (B Lymphphocytes)
28	Cell Assay	Mammalian cells
29	Cell Culture Analog	Mammalian cells (lung, liver, others)
30	Cytosensor Microphysiometer	Mammalian cells
31	Epithelial Cell Toxicity Sensor	Mammalian cells (epithelial)
32	Multianalyte Micro- and Nano- Physiometers	Mammalian cells
33	Neurochip Biosensor System	Mammalian cells (neurons)
34	Portable Cell-Based Biosensor	Mammalian cells (cardiomyocytes)
35	Portable Neuronal Microelectrode Array	Mammalian cells (neurons)
36	SOS Cytosensor System	Fish cells (chromatophores)
37	ToxiLight	Mammalian cells
38	C. *elegans* Monitor	Multicellular: Nematode (*Caenorhabditis elegans*)
39	Instant Multicellular Organisms	Multicellular (Fish aquatic invertebrates, and algae with a life cycle resting stage)
40	IQ Toxicity Test	Multicellular: Daphnid (*Daphnia magna*)

APPENDIX E

EXAMPLE OF AN ENVIRONMENTAL SENTINEL BIOMONITOR TECHNOLOGY FACT SHEET

Microtox, Deltatox

(1) Technology description
  a. Vendor
     Strategic Diagnostics Inc.
     111 Pencader Drive, Newark DE 19702-3322 USA
     Sales: (800) 544-8881 Tel: (302) 456-6789 Fax: (302) 456-6782
     (http://www.sdix.com/ProductSpecs.asp?nProductID=7)
  b. Toxicity sensor type
     i. Living system used: *Vibrio fischeri* (luminescent bacteria)
     ii. Endpoint monitored: Inhibition of light output or toxicity threshold; light is produced as a byproduct of cellular respiration.
     iii. Monitoring method
        1. Freeze-dried bacteria are reconstituted in salt solution containing water sample to be tested
        2. Luminescence readings are taken prior to and at 5 and 15 min. after water sample addition
     iv. Continuous monitoring or grab sample? Grab sample
     v. Note: Microtox and Deltatox generally produce similar results; Deltatox is designed to be field portable and lacks the temperature control capabilities of Microtox.

(2) Logistical considerations
  a. Environmental requirements: Testing done in salt water solution; temperature is maintained at 15 deg. C; ambient temperatures must be 15-30 deg. C.
  b. Storage and transport: Shelf life of freeze-dried bacteria 1 year when stored at -20 to -25° C; less at normal refrigerator temperatures.
  c. Sample throughput: Microtox: 15 samples per hour; Deltatox: 20 per hour
  d. Cube and weight: Microtox: 1.0 ft^3; 21 lbs; Deltatox: 0.2 ft^3, 6 lbs

(3) Response to toxic chemicals (EPA ETV report available)
  a. Chemicals tested: extensive database; see http://www.sdix.com/pdf/Toxicity%20Data%20Index.pdf; also tested as part of MEIC in vitro cytotoxicity evaluation program; see graphs on next page
  b. Toxicity data:
     i. ETV results: no inhibition up to human lethal levels for botulinum toxin, ricin, soman, and VX; aldicarb, colchicine, cyanide, dicrotophos, and thallium were detected at or below lethal levels
     ii. Response relative to human lethal values and MEGs: See graph on next page
  c. Test precision: Range of test standard deviations from ETV evaluation were most less than 10%.
  d. Interfering substances and water conditions:

i. Samples must be dechlorinated before testing; chloraminated water may interfere after dechlorination
ii. Turbid and colored samples may require pre-treatment; pH outside range 6.0 – 8.0 requires adjustment
iii. ETV testing showed copper and zinc to cause interferences

Literature Cited

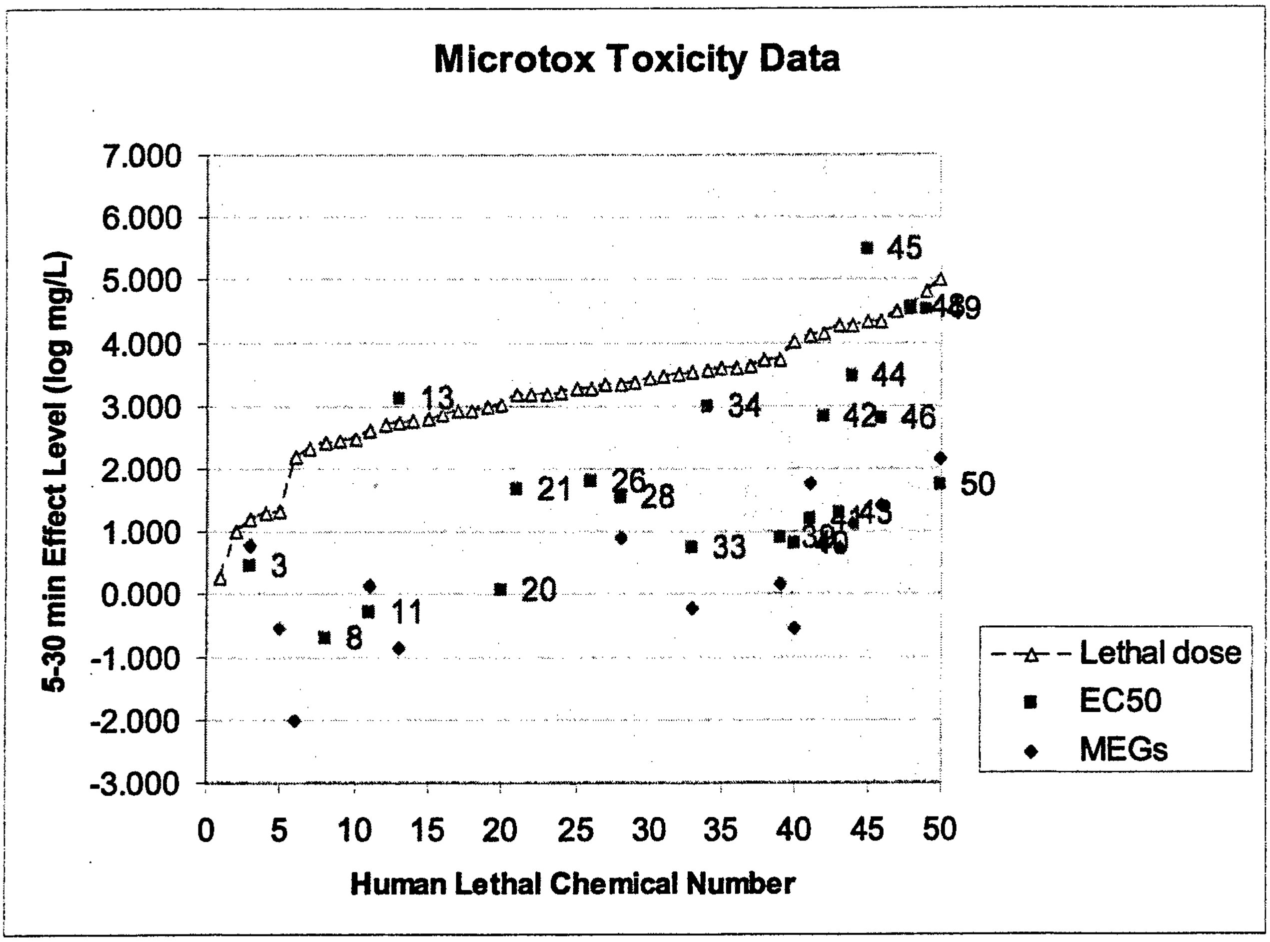
Clemedson C, McFarlane-Abdulla E, Andersson M, Barile FA, et al. 1996. MEIC evaluation of acute systemic toxicity Part I. Methodology of 68 *in vitro* toxicity assays used to test the first 30 reference chemicals. *ATLA* 24:251-272.

Clemedson C, Andersson M, Aoki Y, Barile FA, et al. 1998. MEIC evaluation of acute systemic toxicity Part IV. *In vitro* results from 67 toxicity assays used to test reference chemicals 31-50 and a comparative cytotoxicity analysis. *ATLA* 26:131-183.

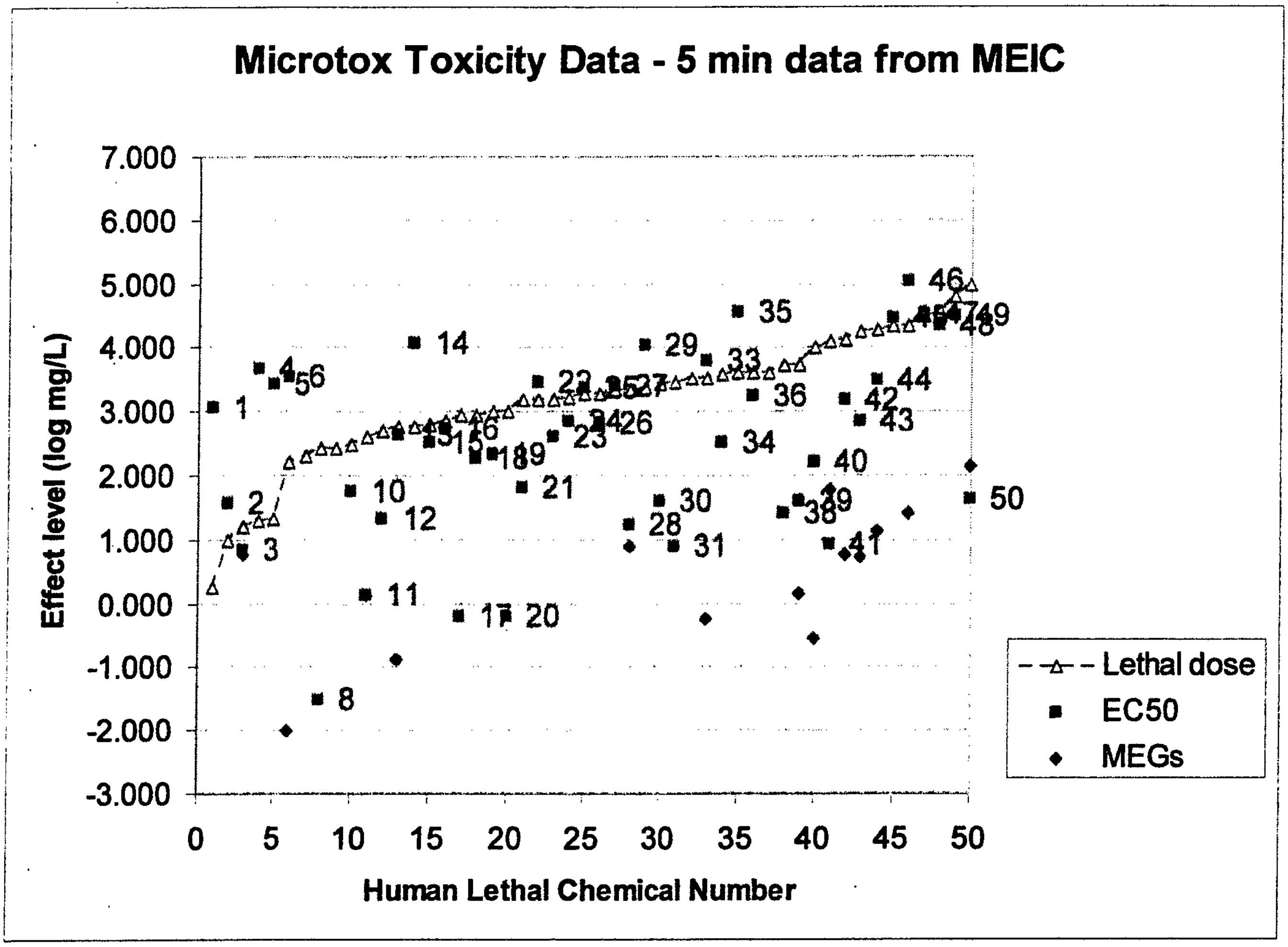
Kaiser KLE, Palabrica VS. 1991. *Photobacterium phosphoreum* toxicity data base. *Water Pollution Research Journal Canada* 26:361-431.

U.S.Army Center for Health Promotion and Preventive Medicine. 2001. Chemical Exposure Guidelines for Deployed Military Personnel. TG-230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.

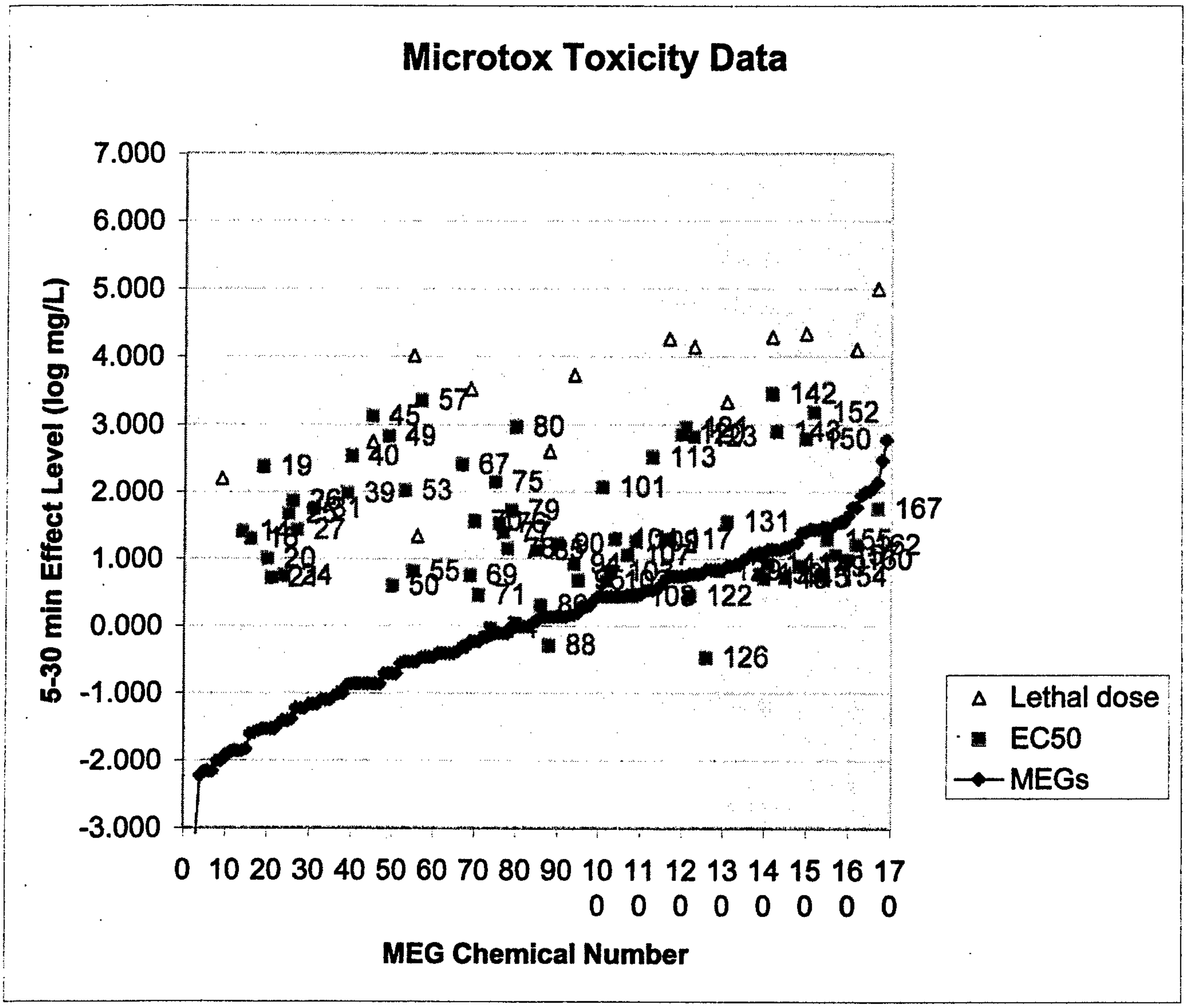
Walum E. 1998. Acute oral toxicity. *Environmental Health Perspectives* 106:497-503.
Ref Type: Journal



Lethal doses for 50 chemicals (Wallum, 1998) are plotted in order of increasing toxicity as concentrations in water, assuming a 70 kg person consuming 5 L of water. Military Exposure Guidelines (MEGs) for water (5 day, 5 L/day) are available for 15 of the 50 chemicals (USACHPPM, 2001). Microtox data from Kaiser and Palabrica, 1991.



Lethal doses for 50 chemicals (Wallum, 1998) are plotted in order of increasing toxicity as concentrations in water, assuming a 70 kg person consuming 5 L of water. Military Exposure Guidelines (MEGs) for water (5 day, 5 L/day) are available for 15 of the 50 chemicals (USACHPPM, 2001). Microtox data from Clemedson et al., 1996, 1998.



Military exposure guidelines (MEGs) for water (5 day exposure, 5 L/day consumption) for 169 chemicals (USACHPPM, 2001) are plotted in order of increasing toxicity. Corresponding human lethal concentrations derived from lethal dose levels (Wallum, 1998) are available for 15 of the 169 chemicals and assume a 70 kg person, 5 L consumption. Microtox data from Kaiser and Palabrica, 1991.

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APPENDIX F

EVALUATION DEFINITIONS AND METHOD FOR THE ENVIRONMENTAL SENTINEL BIOMONITOR QUALITIATIVE SCREENING MODEL

Evaluation Method: A two-step process will be used. The first step is to reduce the number of technologies for further consideration if they do not meet a minimum requirement. The second step is to then assess and then rank the remaining technologies (or top 20) against fundamental criteria.

Step 1: Rate each ESB technology using the rating categories below.

Rating Categories:

Green – means the technology is promising. Final rating and ranking will be completed in step 2.

Orange – means the technology may be promising at some point but (and) the technology is currently immature and needs more R&D (e.g., the technology is not to the point in development where testing can be completed to determine toxicity sensitivity). The technology will not be considered in step 2 but is put on a technical watch list.

Red – means the technology is not promising or not reasonably projected to ever be promising. A technology earns a red rating for any of the reasons below and is eliminated from further consideration.

- Technology toxicity sensitivity is inappropriate or the technology does not provide a unique end-point (i.e., metabolic, physical response).
- Technology is redundant and inferior to another technology.
- Technology is unfeasible/not viable. It does not or is not expected to meet a minimum requirement of a user scenario.
    - Technology takes too much time (hours) to produce an end-point.
    - Technology requires pre-culturing (e.g., requires actively taking out and culturing organism for a long period of time)

Step 2: Evaluate all technologies that were rated green in step 1. Rate each ESB technology against the criteria and rating categories below. After each technology is rated then rank the technologies (1-n).

Rating Categories:

Green – means the ESB technology is known to or is expected to perform "well" against this criterion's requirements or is "likely" to achieve this criterion's requirements.

Yellow – means the ESB technology is known to or is expected to perform only "ok" against this criterion's requirements or "may" achieve this criterion's requirements.

Red – means the ESB technology is known to or is expected to perform "poorly" against this criterion's requirements or is "not likely" to achieve this criterion's requirements.

Evaluation Criteria (see note 1):

- Technology provides an appropriate toxicity sensitivity response (SR) and/or provides unique information (e.g., unique end-point).
- Technology is only minimally affected by interferences (I). Interferences are turbidity, color, and ionic composition of the media.
- Technology is reliable/repeatable (R&R).
- Technology has an appropriate rapidity of response (RoR). A green rating is 20 minutes or less, yellow is 20-60 minutes, and red is over 60 minutes.

Note 1: Ranking decisions are based on the ability of the technologies to meet at least one of the user scenarios. The focus is primarily on appropriate toxicity response – at the correct level of sensitivity, in a reasonable period of time, and with the reproducibility and absence of interferences necessary to reach an appropriate evaluation of a test sample.

APPENDIX G

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating SR	I	R&R	RoR	Step 2 Rationale and Comments
1a	Aquanox	Enzyme (horse-radish peroxidase)	Enzyme	Green-Go to Step 2	Appears to be redundant to Eclox - expert panel is unsure whether there are any advantages that would warrant it's inclusion in the program. Need to verify which of these two systems is preferred.	Green	Green	Green	Green	SR- too sensitive for some chemicals; I - very sensitive to cations and conductivity; R&R- CV less than 10% ; RoR- 5 minutes.
1b	Eclox	Enzyme (horse-radish peroxidase)	Enzyme	Green-Go to Step 2	Developed for military, ready to go now (see comments for Aquanox)	Green	Green	Green	Green	See comments for Aquanox.
2	Microtox, Deltatox	Bacteria (*Vibrio fischeri*)	Bacteria	Green-Go to Step 2	Commercial development and acceptance, widely used, history, lots of data available, field deployable unit available.	Green	Green	Green	Green	SR- more data is available for this than any other assay, with demonstrated sensitivity, but it missed some targets in ETV testing; I - minimally affected by interferences; R&R- false positives will be an issue; RoR- 5-15 min.
3	Mitoscan (Electron Transfer [ETR] and Reverse Electron Transfer [RET])	Enzyme (sub-mito-chondrial particles)	Enzyme	Green-Go to Step 2	Mammalian system may provide some unique sensitivities.	Green	Yellow	Green	Green	SR- one of the best technologies under review; I - expected to be similar to Aquanox, but no data available; R&R- thought to be high, SMPs; RoR- range of 5-20 minutes

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating SR	Step 2 Rating I	Step 2 Rating R&R	Step 2 Rating RoR	Step 2 Rationale and Comments
4	ToxScreen	Bacteria (*Photobacterium leiognathi*)	Bacteria	Green-Go to Step 2	Good complement to Microtox (different bacterium with possible sensitivity differences)?	Green	Green	Green	Yellow	SR- more sensitive than Microtox in ETV testing; two buffer system required (one for metals and one for organics); I - about the same as Microtox ; R&R- within expected range for other similar tests based on ETV testing; RoR- 30-60 minutes.
5	*Sinorhizobium meliloti* Toxicity Test	Bacteria (*Sinorhizobium meliloti*)	Bacteria	Green-Go to Step 2	Similar sensitivity as Microtox, but different toxicity endpoint	Green	Green	Green	Green	SR- most chemicals were detected within the target range; I - divalent cations is the main issue that has to be addressed (this is treatable); R&R- less than 25% CV in many cases; RoR- 20 min. or less.
6	SOS Cytosensor System	Fish cells (chromatophores)	Mammalian cells	Green-Go to Step 2	Requires calibrant; lacks useful data (even though many chemicals have been tested).	Yellow	Yellow	Green	Yellow	SR- Not much data available. Did well at DARPA monitoring workshop for a variety of toxic chemicals; classification scheme available; I - An epithelial cell system that should be less susceptible to matrix-related interferences than mammalian cell systems ; R&R- estimated at +/- 30% ; RoR- Data reported from 1-100 minutes exposure; 60 min should not be a problem.
7	Portable Neuronal Micro-electrode Array	Mammalian cells (neurons)	Mammalian cells	Green-Go to Step 2	Concern with system's high sensitivity to electrolytes (calcium, magnesium) and endotoxin.	Yellow	Red	Green	Yellow	See comments for Portable Cell-based Sensor technology (Tech #32). Need to consider significance of endpoints (cardiac vs. neuronal).

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating SR	I	R&R	RoR	Step 2 Rationale and Comments
8	Portable Cell-Based Biosensor	Mammalian cells (cardiomyo-cytes)	Mammalian cells	Green-Go to Step 2	Concern with system's high sensitivity to electrolytes (calcium, magnesium) and endotoxin.	Yellow	Red	Green	Yellow	SR- many unknowns, what is known is not compelling (but may offer unique detection for certain chemicals and endpoints not found in non-mammalian systems); I - high sensitivity to electrolytes (calcium, magnesium) and endotoxin ; R&R- expected to be good; c.v.s of 20%; RoR- 30 min.
9	Cellsense	Bacteria, algae	Bacteria	Green-Go to Step 2	Cannot be used with substances that are electrochemically active (e.g., the organophosphorus pesticide chlorfenvinphos); would need to check for the presence of this type of chemical by using electrodes without the associated immobilized bacteria.	Green	Green	Yellow	Yellow	SR- data is limited, what is available falls within the MEG/lethal concentration range; I - turbidity and color ok, but there may be issues with electrochemical activity; R&R- screen printed electrodes reduce reproducibility; RoR- 30 min or less
10	Amtox	Bacteria	Bacteria	Green-Go to Step 2	Must add ammonia to influent line; immobilized bacteria; not portable, but within minimum size requirements; capability for continuous monitoring.	Yellow	Yellow	Yellow	Green	SR- sensitivity will be variable; I - no information found; R&R- no information found; RoR- concentration dependent, but may be on the order of a 5-20 minute response cycle.
11	Toxi-Chromo Test	Bacteria (*Escherichia coli*; heavy metal sensitive variant)	Bacteria	Green-Go to Step 2	If mutant, it's rated Red. Unique pathway (gene expression)?	Yellow	Yellow	Yellow	Red	SR- Reported to be less sensitive than Microtox; I - no info available, but colormetric so turbidity will be a problem; R&R- no info available, but probably reproducible (E coli) but thought to be similar to Microtox; RoR- 90 minutes.

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating SR	I	R&R	RoR	Step 2 Rationale and Comments
12	Fluotox	Algae (*Scendedesmus subspicatus*)	Algae	Green-Go to Step 2	Fluotox is the best of the algal based systems, none of which is particularly compelling.	Red	Yellow	Yellow	Yellow	SR- probably most sensitive to herbicides, but may have limited sensitivity to everything else; I - Optical technique, so turbidity is a concern; R&R relying on vendor data; RoR- continuous monitor, so concentration dependent.
13	ArrayScan HCS System	Mammalian cells	Mammalian cells	Green-Go to Step 2	This will not be a standalone technology for the ESB mission - it is a benchmarking tool. There may be a database associated with this system that would be useful for identification of cellular targets. Need more info.	Yellow	Yellow	Green	Red	SR- lacking information, and will require sophisticated lab resources (not field deployable); I - lacking information; R&R- will be within 15-20% range; RoR- over 60 minutes.
See Note 1	Epithelial Cell Toxicity Sensor	Mammalian cells (epithelial)	Mammalian cells	Green-Go to Step 2	SBIR program will provide data that won't cost the ESB STO.	Red	Yellow	Yellow	Yellow	SR- very little data (limited to surfactants that attack the cell wall), no good comparison to other technologies, expected to be poor with 30 minute exposure (cell does not have time to manifest response); I - expected to be similar to other mammalian cell systems; R&R- similar to other mammalian cell systems; RoR- 30 minutes.
	Stressor-Specific E. coli Sensor	Bacteria (*Sinorhizobium meliloti*)	Bacteria	Orange - Tech Watch	Potential specificities for different modes of toxic action. Multiple engineered strains.					

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating: SR	Step 2 Rating: I	Step 2 Rating: R&R	Step 2 Rating: RoR	Step 2 Rationale and Comments
	Cell Culture Analog	Mammalian cells (lung, liver, others)	Mammalian cells	Orange - Tech Watch	This project would be a good marker to watch other developments in this field.					
	Multianalyte Micro- and Nano-Physio-meters	Mammalian cells	Mammalian cells	Orange - Tech Watch	Lots of potential, but deep in R&D and will not be available for several years.					
	C. elegans Monitor	Multicelleular: *Nematode* (*Caenorhabditis elegans*)	Multicellular	Orange - Tech Watch	No evidence that system will ever be sensitive enough. Others (NIEHS) are investigating C. elegans as an environmental monitoring system.					
	Instant Multicellular Organisms	Multicelleular (Fish aquatic invertebrates, and algae with a life cycle resting stage)	Multicellular	Orange - Tech Watch	Potential issues - absorption, identifying endpoint, and enzyme activity.					
	Aqua-Sentinel	Algae	Algae	Red - Eliminated	Reliability/availability of test organisms is a concern.					

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating: SR	Step 2 Rating: I	Step 2 Rating: R&R	Step 2 Rating: RoR	Step 2 Rationale and Comments
	Lumitox	Algae (*Pyrocystis lunula*)	Algae	Red - Eliminated	Reliability/availability of test organisms is a concern					
	Baroxy-meter	Bacteria	Bacteria	Red - Eliminated	Skeptical about respiration as an endpoint. Concern with matrix effects - *it may be necessary to match nutrient* (organic) levels between the unknown sample and the reference sample to allow comparisons of respiration rate.					
	BioTox Flash Test	Bacteria (*Vibrio fischeri*)	Bacteria	Red - Eliminated	Deltatox and Microtox would be more promising Vibrio fischeri based approaches, especially considering available sensitivity test results.					
	Green-Screen EM	Yeast (*Saccharomyces cerevisiae*)	Bacteria	Red - Eliminated						
	LUMIStox	Bacteria (*Vibrio fischeri*)	Bacteria	Red - Eliminated	Deltatox and Microtox would be more promising Vibrio fischeri based approaches, especially considering available sensitivity test results.					
	MetPlate	Bacteria (*Escherichia coli*)	Bacteria	Red - Eliminated	Limited scope of detection (just metals); requires 4-5 hr incubation period.					

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating SR	I	R&R	RoR	Step 2 Rationale and Comments
	Polytox	Bacteria	Bacteria	Red - Eliminated	Skeptical about respiration as an endpoint. Concern with matrix effects - it may be necessary to match nutrient (organic) levels between the unknown sample and the reference sample to allow comparisons of respiration rate.					
	STIPTOX	Bacteria	Bacteria	Red - Eliminated	See comments for Baroxy-meter					
	ToxTrak	Bacteria	Bacteria	Red - Eliminated	Best results are with cultures aged 10 to 72 hours.					
	Vitotox	Bacteria (*Salmonella tyhimurium*)	Bacteria	Red - Eliminated	Extensive test time (3 hrs), prep time (overnight).					
	Brain on a Chip	Mammalian cells	Mammalian cells	Red - Eliminated	Not practical - tissue can't be kept alive for longer than 12 hrs.					
	CANARY Biosensor	Mammalian cells (B Lymphpho-cytes)	Mammalian cells	Red - Eliminated	Turn over technology to JSAWM project for further consideration					
	Cell Assay	Mammalian cells	Mammalian cells	Red - Eliminated	Vendor non-responsive; sensor, no bio. Capturing cell in microfluidic system is impractical.					

SCREENING MODEL RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM TECHNOLOGIES

Rank	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating SR	I	R&R	RoR	Step 2 Rationale and Comments
	Cytosensor Microphysiometer	Mammalian cells	Mammalian cells	Red - Eliminated	Not a stand-alone technology. End point is the measure of a calls viability.					
	Neurochip Biosensor System	Mammalian cells (neurons)	Mammalian cells	Red - Eliminated	Technology gets folded into Portable Neuronal Microelectrode Array (Tech #33)					
	ToxiLight	Mammalian cells	Mammalian cells	Red - Eliminated	Not a stand-alone technology. End point is the measure of a cells viability.					
	IQ Toxicity Test	Multi-cellular: Daphnid (*Daphnia magna*)	Multicellular	Red - Eliminated	Too sensitive to be of practical use, takes too long, daphnids are logisitically difficult to work with.					

Note 1: Funded under a different program

Step 1 Ratings
Green-Go to Step 2
Orange - Tech Watch
Red - Eliminated

Step 1 Reasons for Eliminating
Inappropriate Sensitivity
Redundant & Inferior
Infeasible-time
Infeasible-pre-culturing
Infeasible-other
Other
Open

Step 2 Rating
Green
Yellow
Red

APPENDIX H

INFORMATION ON SELECTING TRAINING/TEST CHEMICALS AND LIST OF TRAINING/TEST CHEMICALS

Information on Selection of Training/Test Chemicals:

Considering the thousands of toxic industrial and agro-chemicals, an appropriate evaluation of toxicity sensor performance requires careful initial selection of the chemicals to be evaluated. Some possible criteria for selecting test chemicals include:

- Threat chemicals. Several organizations (U.S. Environmental Protection Agency (EPA), U.S. Army Research Development and Engineering Center (ARDEC)) and publications (Garland, 1991; Hickman, 1999; Clark and Deininger, 2000) have developed lists of threat chemicals for water supplies. Typically, the higher priority chemicals are highly toxic, soluble, persistent in water, and available in the region of concern. Chemicals high on these lists should be given priority as test chemicals for toxicity sensors.
- Mode of toxic action. Some chemicals can be grouped together because they cause similar effects on organisms. Examples include organophosphorus and carbamate insecticides (which cause acetylcholinesterase inhibition) and many neutral organic chemicals such as industrial solvents (which cause narcosis). To the extent that chemicals can be grouped in this way, it should be possible to select one or two representative chemicals from the group for testing to establish whether a toxicity sensor will respond to chemicals exhibiting the same general mode of toxic action.
- Chemical structural classes. Chemicals with certain structural, functional, or other similarities are frequently grouped together for testing purposes, such as heavy metals, algal toxins, or herbicides. Since chemicals within these groups may have distinctly different modes of toxic action, it is best to emphasize mode of toxic action categories in the selection of test chemicals. The MEIC chemical list provides a broad range of both modes of toxic action and chemical structural classes (Clemedson *et al.*, 1996, 1998).
- Interfering chemicals or water quality conditions. Interfering chemicals include substances commonly found in raw or treated water that may cause toxicity sensor responses at levels far below concentrations affecting humans, such as residual chlorine, copper, or ammonia. In addition, acceptable ranges of common water quality conditions, such as temperature, dissolved oxygen, conductivity, pH, turbidity, and microbial concentrations need to be defined for toxicity sensors to help minimize "false positive" responses.

Given the broad range of potential test substances, a compromise must be reached between minimizing the number of chemicals to conserve resources and expanding the number to better define toxicity sensor response characteristics. One possible solution is the use of a tiered approach – conduct an initial screening of

several toxicity sensor technologies with a few chemicals, followed by more thorough evaluation of technologies showing the most promise.

List of Training/Test Chemicals:

Chemicals are: Aldicarb *, Ammonia, Copper Sulfate, Mercuric Chloride *, Methamidophos *, Nicotine *, Paraquat Dichloride *, Pentachlorophenol *, Phenol, Sodium Arsenite *, Sodium Cyanide *, and Toluene.

Notes: "*" means it is a user-selected chemical

The responses of each toxicity sensor to residual chlorine and a high conductivity freshwater sample will be determined as well.

References

Clark RM, Deininger RA. 2000. Protecting the nation's critical infrastructure: The vulnerability of U.S. water supply systems. *Journal of Contingencies and Crisis Management* 8:73-80.

Garland JG. 1991. Water Vulnerability Assessments. AL-TR-1991-0049. Armstrong Laboratory, Occupational and Environmental Health Directorate, Brooks Air Force Base, TX.

Hickman, DC. 1999. A Chemical and Biological Warfare Threat: USAF Water Systems at Risk. Counterproliferation Paper No. 3. USAF Counterproliferation Center, Air War College, Maxwell Air Force Base, AL.

APPENDIX I

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM: QUANTITATIVE MODEL DESCRIPTION AND DETAILS

Three separate multi-criteria decision-making models were developed. See *Figures I-1* through *I - 3* for a visual representation of the models. The weights for each goal and measure are also detailed in these figures. The models (goals, end-points, utility curves) are described within each of the ten measures below.

Note: The numbers after the measure name refer to the 26 technical requirements the user representatives developed for each scenario. Key: FB – Fixed Base; FC – Field/Contingency; SU - Small Unit

Measure #: 1

Measure Name: Chemical Detection (2)

Goal(s): Performance

Definition: The ability of the technology to provide an adverse response to a set of representative chemicals at concentrations between the short-term MEG and the HLC for the greatest number of test chemicals. For this measure, it does not matter how close the technology provides an adverse response to the MEG.

What's Desired? It is best to respond to all (the best technologies detected 6) chemicals between the short-term MEG and the HLC.

Scale:

FB/FC/SU Scenarios:

100 - Six (6) chemicals detected in the MEG-HLC range

0 - No chemicals detected in the MEG-HLC range

Linear continuous utility curve used

Scenario Specifics: The performance scale and utility function for all 3 scenarios are the same. Weight is lower in FC and SU scenarios than the FB scenario due to willingness to accept more risks in field conditions/situations.

Notes: The scoring scale chosen was selected due to greater level of discrimination provided by this scale. The other scoring scale, which is a combination of the number of chemicals identified in the MEG-HLC concentration range with a calculation of closeness of the sensor response to the MEG, was not selected because the maturity of the sensor technologies did not justify this level of discrimination. Dilution of all samples can be done by calculation for

any technology/sensor that detect consistently below the MEG, after which the score would be recalculated for that technology.

Measure #: 2

Measure Name: Water Characteristics (5,6)

Goal(s): Performance

Definition: The ability to operate under a number of water quality conditions and in the presence of interfering substances with minimal effect on test outcome. Performance scale is based on residual chlorine as the substance that provides the most discrimination between the sensors.

What's Desired? It is better to be able to operate under a wide range of water quality conditions (higher concentrations of residual chlorine rather than lower levels) than under a more restricted range.

Scale:

FB/FC/SU Scenarios:

100	- > 5 mg/L residual chlorine
75	- > 2-5 mg/L residual chlorine
50	- 1-2 mg/L residual chlorine
0	- < 1 mg/L residual chlorine

Discrete levels

Scenario Specifics: The performance scale and utility function for all 3 scenarios are the same. Having to dechlorinate or make other water quality adjustments for the FB scenario is not as significant as having to make adjustments for the SU scenario.

Notes:

No particular water characteristics are more important than others.

Water characteristics previously identified for consideration: quantity of total dissolved solids, turbidity, clay, chlorine residual, ammonia, and metals [e.g., copper], pH, temperature, cations, chloramines, and coagulants.

Of these, most ESB technologies that require temp or pH control include a temp/pH control mechanism. Turbidity is not a discriminator, as no ESB technology will operate properly in turbid water (the water must be filtered). Blind sample test data are available for total dissolved solids and cations (tested very high hardness water), residual chlorine (up to 10 mg/L TRC), ammonia, and copper. There was only one response below the MEG to copper and no responses below the MEG to ammonia. Only the S. meliloti system responded to the high hardness water sample. Preliminary data

suggest only chlorine has any discriminatory ability (at least half the sensors responded at concentrations below 10 mg/L). Note that residual chlorine may be removable by adding a reducing agent such as sodium bisulfite, but could affect sensor response to other chemicals or change the nature of the target contaminant.

The ability of ESB technologies to perform in water with Humic acid, Fulvic acid, and additional disinfectants may be an important discriminator. Consider testing ESB Systems with these interfering substances in the future.

Measure #: 3

Measure Name: Technology Failure Rate (16)

Goal(s): Performance

Definition: The number of times a technology fails to produce viable/usable result per a certain number of tests. This measure does not quantify failure because of a human mistake. The technology being evaluated includes the biological basis of the system and all ancillary equipment.

The calculation for the failure rate is: [(number of tests attempted) - (number of tests completed)]/ (number of tests attempted)] x 100%

What's Desired? It is better to have fewer rather many failures.

Scale:

FB Scenario

100	- 0% failure rate
67	- > 0-5% failure rate
33	- > 5-15% failure rate
0	- > 15% failure rate

Discrete levels

FC Scenario:

100	- 0% failure rate
80	- > 0-5% failure rate
30	- > 5-15% failure rate
0	- > 15% failure rate

Discrete levels

SU Scenario:

100	- 0% failure rate
85	- > 0-5% failure rate
20	- > 5-15% failure rate
0	- > 15% failure rate

Discrete levels

Scenario Specifics: The performance scale and utility function for all 3 scenarios are slightly different. Weight is lower in FC and SU scenarios than the FB scenario due to willingness to accept more risks in field conditions/situations.

Notes: The best comparative data for this metric is the data provided by the toxicity sensor testers. They have calculated their failure rate (using equation above in definition) for their tests. In the next phase of testing it's important to determine the four potential sensor responses for each technology (True positives, True negatives, False positives, False negatives). More testing required for project to transition to next milestone.

Measure Measure #: 4

Measure Name: Test Reproducibility (19)

Goal(s): Performance

Definition: The ability of the ESB technology to produce the same output/answer given the same input and test conditions over multiple tests. This is measured as median coefficient of variation; a low coefficient of variation represents high reproducibility.

What's Desired? It is best to have high reproducibility (a low coefficient of variation).

Scale:

FB/FC/SU Scenarios:

100 - 6% median coefficient of variation
0 - 54% median coefficient of variation

Nonlinear continuous curve.

Scenario Specifics: The performance scale and utility function for all 3 scenarios are the same. Weight is lower in FC and SU scenarios than the FB scenario due to willingness to accept more risks in field conditions/situations.

Notes: User representatives recommend giving those sensors with no data available a score of zero.

Measure Measure #: 5

Measure Name: Test Turn Around Time (22, 23)

Goal(s): Operational Impact

Definition: The time required between consecutive tests (assumes reusable technology). Includes operator set-up time, sample preparation, sensor operation time, and any time required for the system to reset before another reading.

What's Desired? It is better to have less rather than longer time required between consecutive tests.

Scale:
FB/FC/SU Scenarios

100 - 5 minutes
0 - 180 minutes

Nonlinear continuous curve.

Scenario Specifics: The performance scale and utility function for all 3 scenarios are the same.

Measure #: 6

Measure Name: Sample Preparation and Skills Required (21, 25, 26)

Goal(s): Operational Impact

Definition: The sample preparation, performing test complexity level (e.g., measuring volume, adding reagent), and location of analysis (at sample site vs. in a lab). The focus is not on how long it takes to perform each task but rather how complicated each task is.

What's Desired? It is better for the tasks to be simple (e.g., a soldier can perform) rather than complex (e.g., a technician must perform at a depot).

Scale:
FB Scenario

100 - few steps of preparation, minimal skill level required (E4, 91S), analysis done at sample site
67 - some steps of preparation, moderate skill level (NCO and above, water technician with lab skills and lab capabilities)
33 - many steps of preparation, significant skill level required (senior lab technician with lab skills and lab capabilities)
0 - many steps of preparation, expert skill level (e.g.; research scientist) required

Linear continuous curve

FC Scenario:

100 - few steps of preparation, no skill level required (E2, any MOS), analysis done at sample site

90 - few steps of preparation, minimal skill level required (E4, 91S), analysis done at sample site
50 - some steps of preparation, moderate skill level (NCO and above, water technician with lab skills and lab capabilities)
0 - many steps of preparation, significant skill level required (senior lab technician with lab skills and lab capabilities)

Linear continuous curve

SU Scenario:

100 - few steps of preparation, no skill level required (E2, any MOS), analysis done at sample site
70 - few steps of preparation, minimal skill level required (E4, 91S), analysis done at sample site
0 - some steps of preparation, moderate skill level (NCO and above, water technician with lab skills and lab capabilities)

Linear continuous curve

Scenario Specifics: The performance scale and utility function for all 3 scenarios are different. Weight is lower in FB scenario than FC and SU scenarios because of the additional manpower and expertise available with the FB scenario.
E2 is an entry level of technical skill. E4 would have some lab training and higher levels of training and experience translates to a senior lab technician who might be an E6, E7, or civilian/contractor

Measure #: 7

Measure Name: Weight (8, 11)

Goal(s): Logistics

Definition: The weight of the technology and necessary peripheral equipment (e.g., maintenance and repair supplies and parts, power source, and a 2-week supply of consumables).

What's Desired? It is better for the overall weight to be less rather than more.

Scale:

FB/FC/SU Scenarios:

100 - 1 lb
0 - 40 lb

S-shaped continuous curve

Scenario Specifics: The performance scale is the same for all 3 scenarios but the utility function is different for each scenario. Weight is lower in FB scenario than the FC and SU scenarios as there are more options (e.g., carry in a truck vs. a backpack) and the ability to handle an ESB technology that weighs a lot (40 lbs vs. 1 lb).

Notes: Size will not be considered for this evaluation as weight is considered a surrogate for volume/size.

Measure #: 8

Measure Name: Shelf Life (17)

Goal(s): Logistics

Definition: The storage life of the technology and consumables (i.e., how long will they be usable [includes shipping time] under optimal conditions for that technology).

What's Desired? It is better when the technology and its consumables have a long shelf life.

Scale:

FB Scenarios

100	- greater than 12 months
0	- 2 weeks (1/2 month) or less

Non-linear continuous curve

FC Scenario:

100	- greater than 12 months
75	- 6-12 months
40	- 3-6 months
0	- 3 months or less

Discrete levels

SU Scenario:

100	- greater than 12 months
75	- 6-12 months
30	- 3-6 months
0	- 3 months or less

Discrete levels

Scenario Specifics: The performance scale and utility function for all 3 scenarios are different. Weight is lower in FB scenario than the FC and SU scenarios as there are more options and the ability to handle an ESB technology with a short shelf life.

Notes: Expert input estimates shelf life for ESB technology components to range from 2 weeks to 2 years.

Measure #: 9

Measure Name: Storage Conditions Required (17)

Goal(s): Logistics

Definition: The conditions required to maintain the technology in operating condition for the maximum possible shelf life.

What's Desired? The least special or demanding storage conditions.

Scale:

FB Scenario:

100 - Easy, ambient conditions (includes extremes up to 71 degrees C)
50 - Average, controlled room temperature
30 - Demanding, refrigeration required
0 - Extremely demanding, very low temp or narrow temp range required

Discrete Levels

FC Scenario:

100 - Easy, ambient conditions (includes extremes up to 71 degrees C)
50 - Average, controlled room temperature
0 - Demanding, refrigeration required

Discrete Levels

SU Scenario:

100 - Easy, ambient conditions (includes extremes up to 71 degrees C)
0 - Controlled room temperature

Discrete Levels

Scenario Specifics: The performance scale and utility function for all 3 scenarios are different. Weight is lower in FB scenario than the FC and SU scenarios as there are more options and the ability to handle an ESB system that requires demanding storage condition.

Measure #: 10

Measure Name: Biological Component Maintenance (17)

Goal(s): Logistics

Definition: Time required, complexity of system, and material and logistics required to maintain biological component of the test system.

What's Desired? Requires no biological culturing. Time required to maintain biological component is minimal, system to maintain is simple, and requirements to maintain organism culture is minimal.

Scale:

FB Scenario

100	- requires no biological culturing
60	- requires some maintenance but no culture medium exchange
0	- requires culture medium exchange or CO2 for culture medium

Discrete levels

FC Scenario:

100	- requires no biological culturing
40	- requires some maintenance but no culture medium exchange
0	- requires culture medium exchange or CO2 for culture medium

Discrete levels

SU Scenario:

100	- requires no biological culturing
0	- requires some maintenance but no culture medium exchange

Discrete levels

Scenario Specifics: The performance scale and utility function for all 3 scenarios are different. Weight is lower in FB scenario than the FC and SU scenarios as there are more options and the ability to handle an ESB system that requires biological component maintenance.

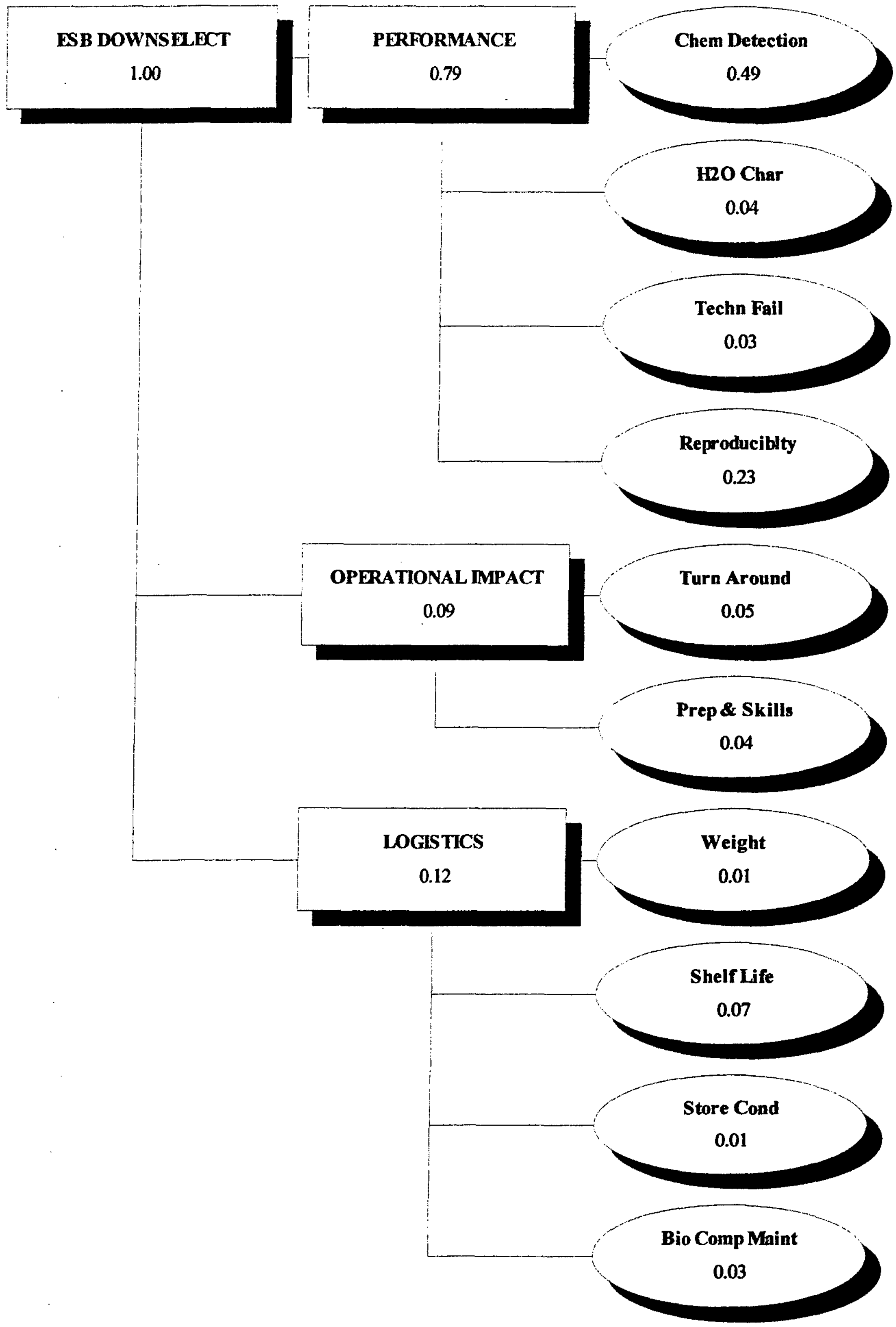

Figure I- 1: Fixed Base Scenario Model with Weights

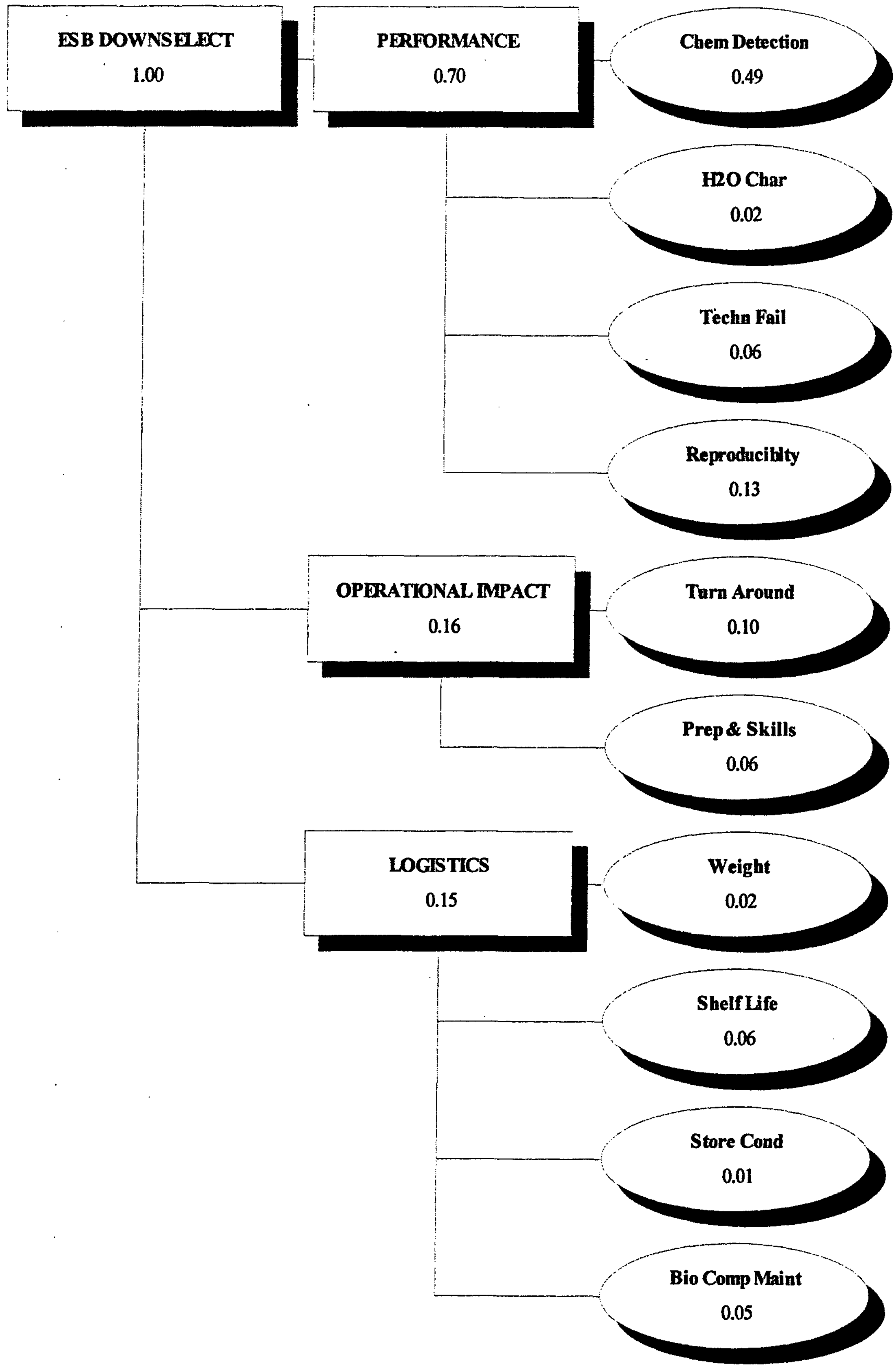

Figure I-2: Field Contingency Scenario Model with Weights

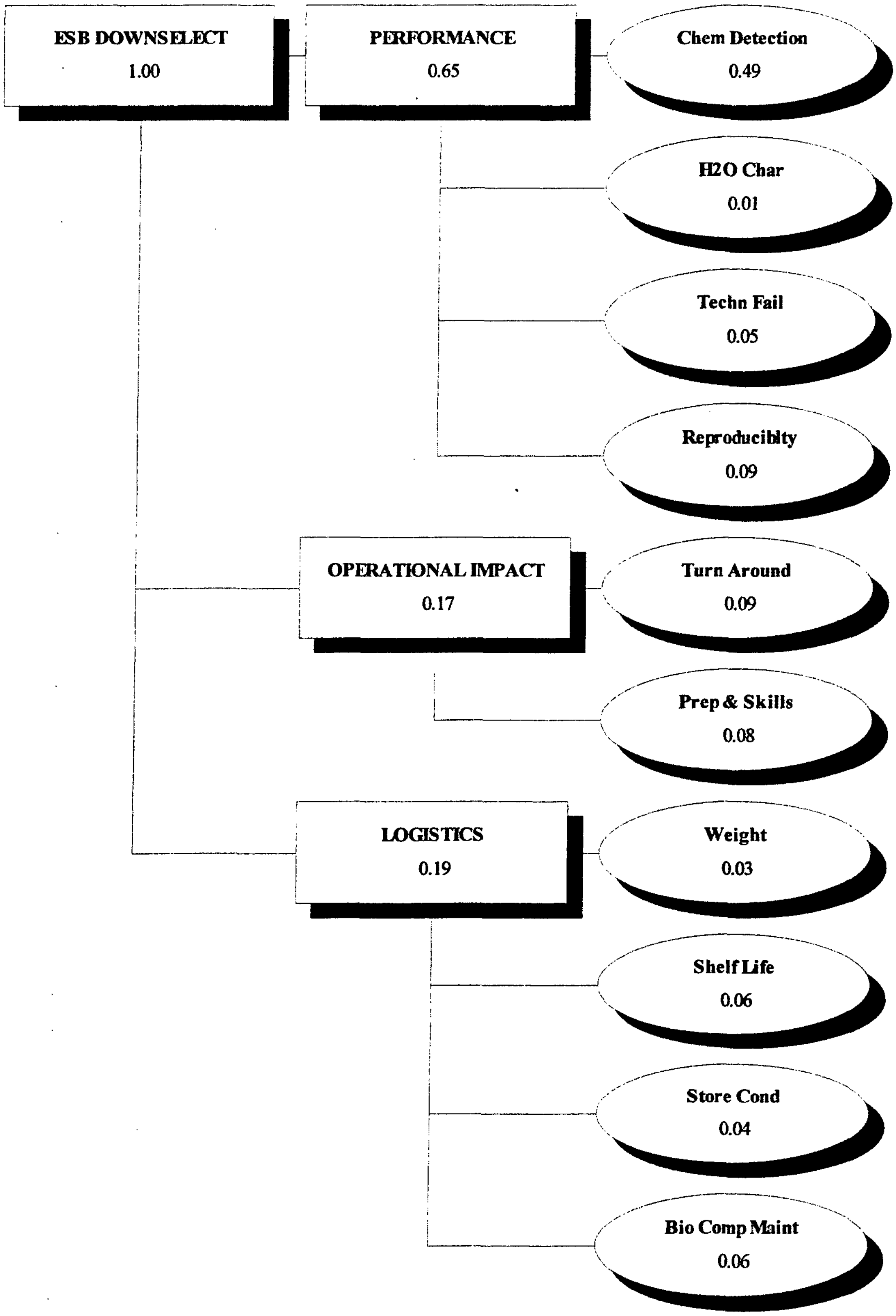

Figure I-3: Small Unit Scenario Model with Weights

APPENDIX J

ENVIRONMENTAL SENTINEL BIOMONITOR PROGRAMMATICS ASSESSMENT			
ESB Technology	**Cost** (see Notes 1,2 & 3)	**Risk** (see Notes 1 & 3)	**Potential for Technology's Capabilities to Improve** (see Notes 1 & 3)
ECIS	Cartridge and cell maintenance system is developmental and likely to be somewhat costly.	Medium risk. Technology still under development.	High potential for miniaturization and to expand cell lines
Hepatocyte LDL	Probably reasonable cost - need to verify.	High risk because technology's not a commercial product yet and technology has not been independently verified (some promising result have been published.)	Significant potential for further improvement.
Microtox	Fairly reasonable for capital investment and maintenance. Some increased costs because of need to maintain cultures.	Low risk. Very mature technology.	Little potential for further development.
Neur Microel Array	Relatively high cost partially due to high consumable costs (e.g., disposable hardware).	High risk because technology in early R&D stage. Not a very mature technology.	Significant potential for further improvement.
Daphnia IQ Test	Fairly reasonable for capital investment and maintenance. Some increased costs because of need to maintain cultures (may be frozen, then thawed when needed).	Fairly low (one technical expert believes there is some research that indicates freezing daphnids is possible-need to confirm).	Potential to reduce maintenance requirements by using frozen and then thawed daphnids.

Note 1: Because technologies are in an early stage of development, because of the limited programmatic data about each technology, and because of the limited time the technical experts had to complete this assignment, the programmatic input/comments are very general.
Note 2: Costs don't include initial technology development costs but rather only costs to initially buy final technology product and maintain it to include supplies to operate it.
Note 3: Maturity level of technology is considered in evaluation.

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APPENDIX K

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM: TECHNOLOGY STRENGTHS AND WEAKNESSES ANALYSIS

The purpose of the technology analysis is to highlight areas where particular technologies stand out, either positively or negatively. This appendix contains narrative summaries (*Table K-1*) of the relative strengths and weaknesses for each technology, as well as charts (*Figures K-1 – K-12*) that show how each technology performed relative to the ten measures.

For the narrative summaries "average/below average" descriptors are used. These were subjectively determined by the DAT and are based on each ESB technology's score relative to the other ESB technologies.

The twelve ESB technologies listed below were evaluated by the DAT against how they scored relative to the Fixed Base Scenario model. They are presented in the order that they scored against the Fixed Base Scenario model (i.e., number in parenthesis is score [e.g., 82 for Microtox]).

Table K-1: Technology Strengths and Weaknesses Analysis Narrative Summaries

1.	Microtox (82) scored above average on seven measures (*Chemical Detection, Technology Failure Rate, Test Turn Around Time, Preparation and Skills Required, Shelf Life, and Biological Component Maintenance*) and below average on *Water Characteristics*, *Weight,* and *Storage Conditions Required* measures. Microtox detected six chemicals, including one chemical that the other three top-ranked technologies did not detect - cyanide.
2.	Hepatocyte Low Density Lipoprotein Uptake (72) scored above average on four measures (*Chemical Detection, Test Reproducibility, Water Characteristics,* and *Biological Component Maintenance*) and below average on all other measures. Hepatocyte Low Density Lipoprotein Uptake detects six chemicals and one chemical the other three technologies in the top four ranked technologies combined don't detect - paraquat.
3.	Electric Cell-Substrate Impedance Sensing (67) scored above average on two measures *(Chemical Detection* and *Water Characteristics)* and below average on *Technology Failure Rate, Test Turn Around Time, Sample Preparation and Skills Required, Weight, Shelf Life, Storage Conditions Required,* and *Biological Component Maintenance.* Electric Cell-Substrate Impedance Sensing detects one chemical, ammonia, that the top four ranked technologies combined do not detect.
4.	Mitoscan Electron Transfer (65) scored above average on six measures (*Water Characteristics, Technology Failure Rate, Test Reproducibility, Weight, Shelf Life, and Biological Component Maintenance*) and below average on *Chemical Detection, Sample Preparation and Skills Required*, and *Storage Conditions Required.* Mitoscan Electron Transfer does not detect any chemicals that the top four ranked technologies combined do not detect.

5.	Eclox (55) scored above average on seven measures (*Technology Failure Rate, Test Reproducibility, Test Turn Around Time, Sample Preparation and Skills Required, Shelf Life, Storage Conditions Required,* and *Biological Component Maintenance*) and below average for *Chemical Detection* and *Water Characteristics* measures. Eclox only detects one chemical - copper. The top four scored technologies also detect copper.
6.	Neuronal Microelectrode Array (52) scored above average for the *Test Reproducibility* measure and below average on six measures (*Technology Failure Rate, Test Turn Around Time, Preparation and Skills Required, Weight, Shelf Life,* and *Storage Conditions Required*). But the Neuronal Microelectrode Array detects two chemicals the top four rated technologies do not detect (aldicarb and methamidophos – organophosphates).
7.	SOS Cytosensor (51) scored above average in three measures (*Chemical Detection, Water Characteristics,* and *Storage Conditions Required*) and below average on *Test Reproducibility, Test Turn Around Time, Preparation and Skills Required,* and *Shelf Life* measures. SOS Cytosensor detects five chemicals although it does not detect any chemicals that the top four rated technologies do not detect.
8.	Toxi-Chromotest (49) scored above average on three measures (*Water Characteristics, Technology Failure Rate,* and *Shelf Life*) and below average on *Chemical Detection, Test Turn Around Time,* and *Storage Conditions Required.* Toxi-Chromotest only detects two chemicals and these chemicals are not different from the chemicals detected by the highest four scored technologies.
9.	ToxScreen Organic (tied with ToxScreen Metal, 42) scored above average on three measures (*Preparation and Skills Required, Weight, and Shelf Life*) and below average on *Water Characteristics, Technology Failure Rate, Test Reproducibility, and Test Turn Around Time.* The three chemicals detected are not different from the chemicals detected by the highest four scored technologies.
10.	ToxScreen Metal (tied with ToxScreen Organic, 42) scored above average on three measures (*Preparation and Skills Required, Weight, and Shelf Life*) and below average on *Technology Failure Rate, Test Reproducibility, and Test Turn Around Time.* The three chemicals detected are not different from the chemicals detected by the highest four scored technologies.
11.	*Sinorhizobium meliloti* Assay (40) scored above average on five measures (*Water Characteristics, Test Turn Around Time, Preparation and Skills Required, Weight and Storage Conditions Required*) and below average on *Test Reproducibility, Shelf Life,* and *Biological Component Maintenance* measures. It detected three chemicals, none that the highest four scored technologies do not also detect.
12.	Mitoscan Reverse Electron Transfer (33) scored above average on four measures (*Technology Failure Rate, Weight, Shelf Life,* and *Biological Component Maintenance*) and below average on *Chemical Detection, Preparation and Skills Required, Weight,* and *Storage Conditions.* It detected one chemical, none that the highest four scored technologies do not also detect.

Each chart below, *Figures K-1 – K-12*, represent a different ESB technology. They depict the strengths and weaknesses for each of the 17 ESB technologies relative to the ten measures in the Fixed Base Scenario model. The height of the bars indicates a

technology's relative score for each measure, while the width indicates the relative weight of the measure. For Microtox, for example, the chart shows it scored very high against four measures, which are *Chemical Detection* (highest weighted measure also), *Shelf Life, Biological Component Maintenance,* and *Technology Failure Rate.* The measures where Microtox scored low were generally weighted low, for example the *Water Characteristics* measure (H2O Char, 5th bar from the left). The charts are provided in the order of overall technology ranking results from best to worst (based on Fixed Base Scenario model results).

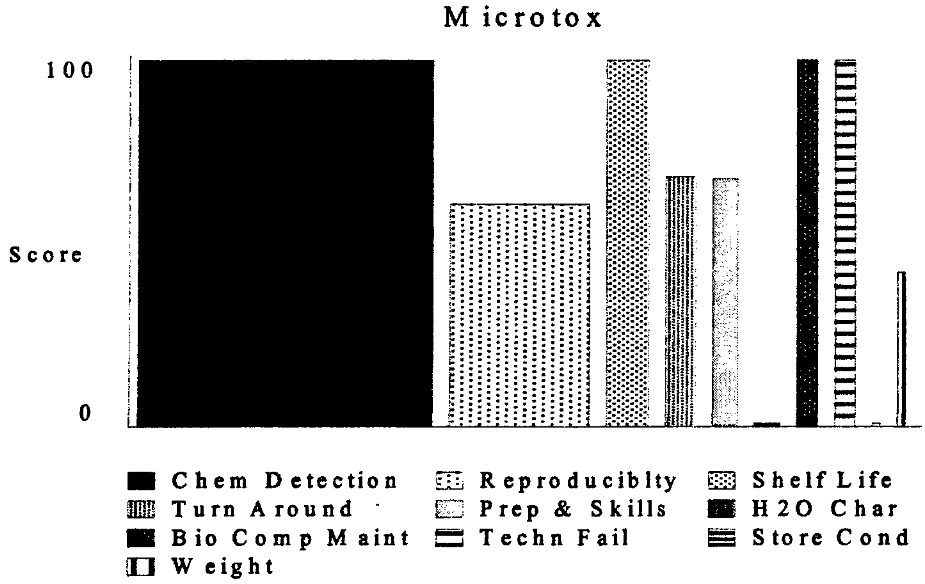

Figure K-1: Microtox Strengths and Weaknesses Chart

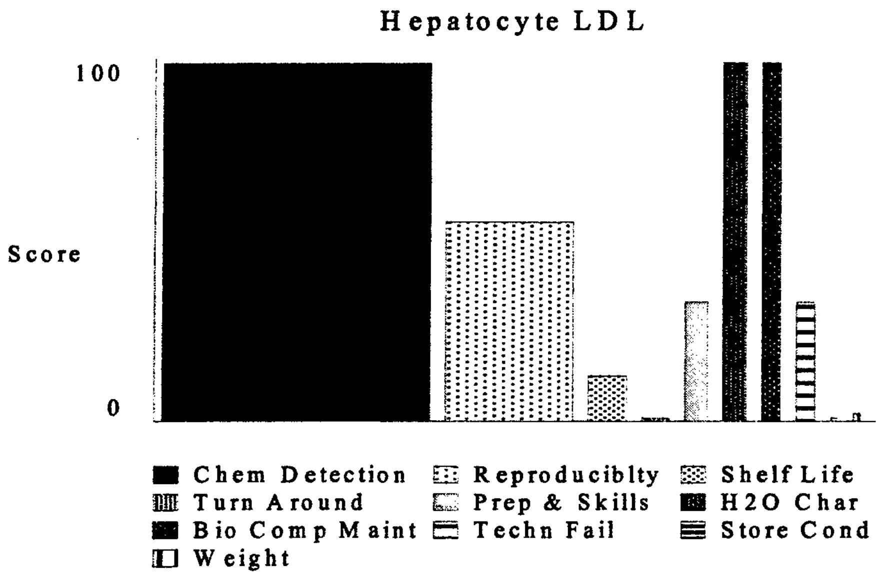

Figure K-2: Hepatocyte Low Density Lipoprotein Uptake Strengths and Weaknesses Chart

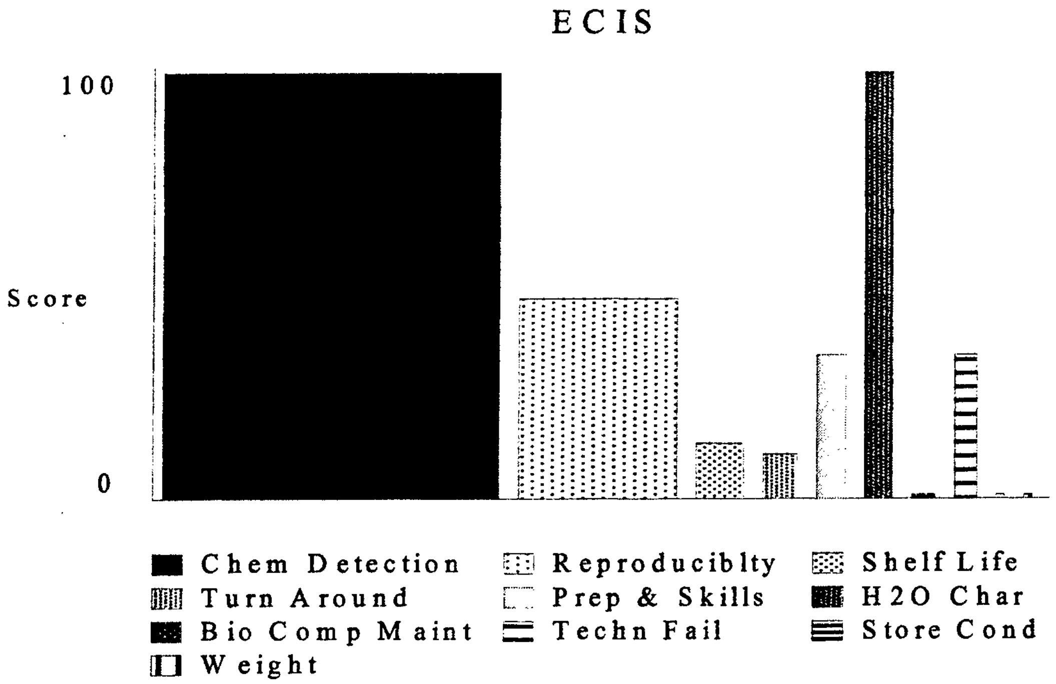

Figure K-3: Electric Cell-Substrate Impedance Sensing Strengths and Weaknesses Chart

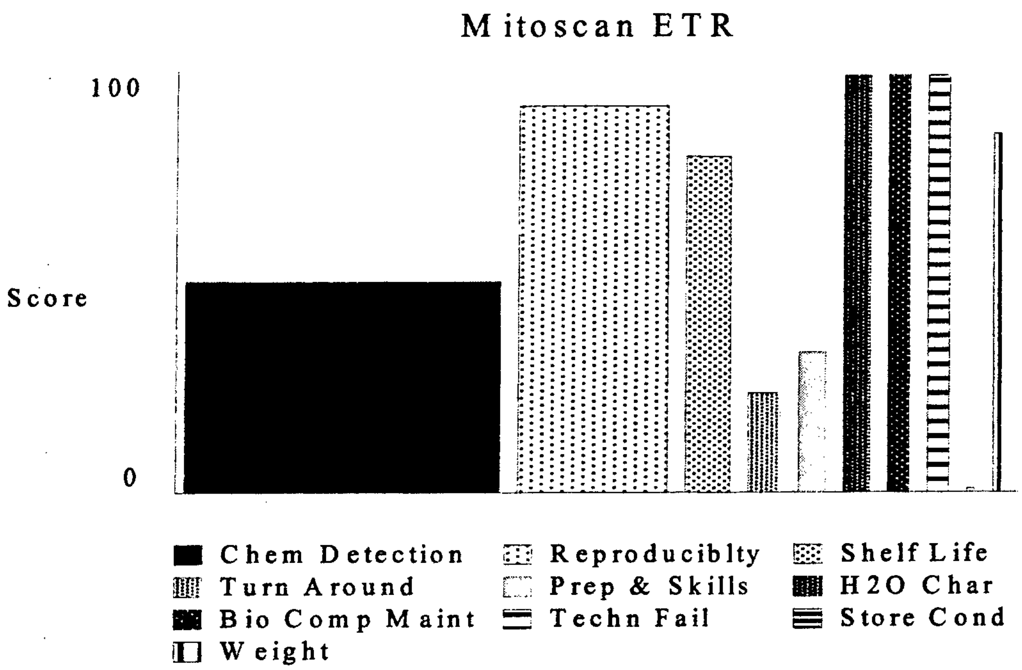

Figure K-4: Mitoscan Electron Transfer Strengths and Weaknesses Chart

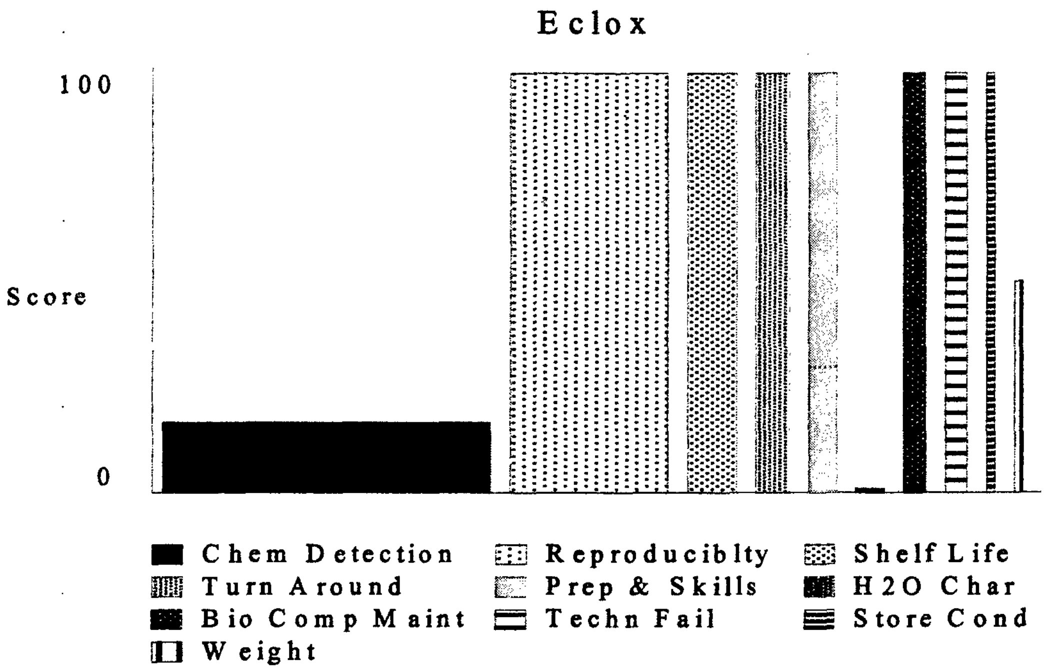

Figure K-5: Eclox Strengths and Weaknesses Chart

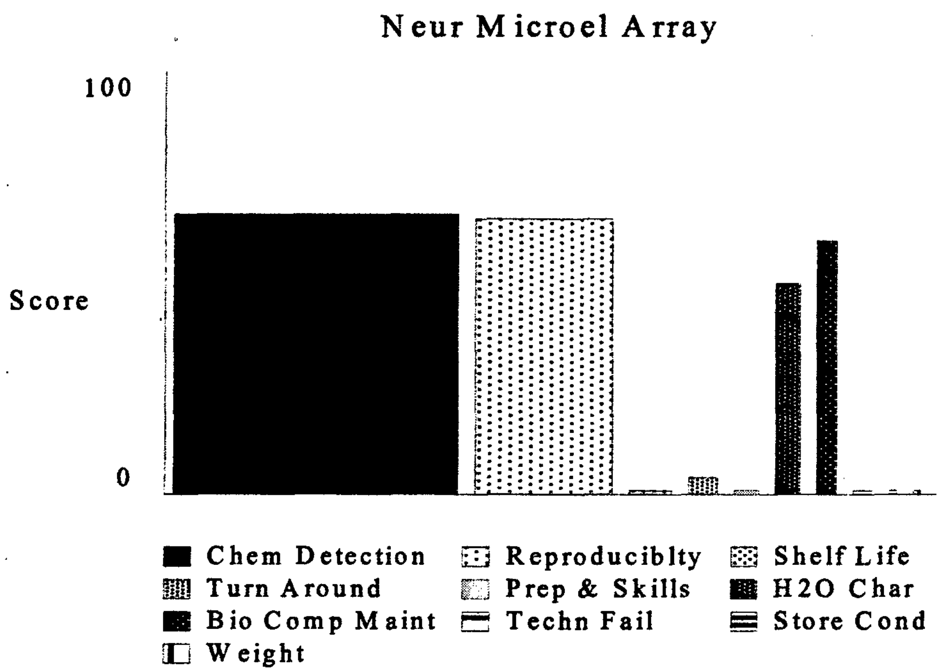

Figure K-6: Neuronal Microelectrode Array Strengths and Weaknesses Chart

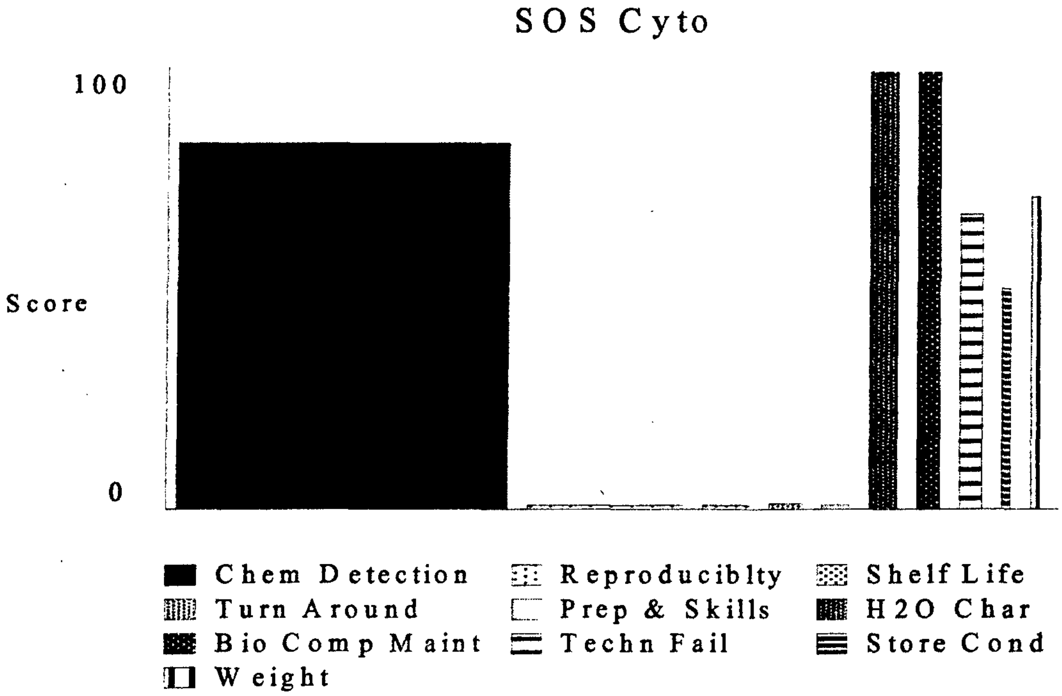

Figure K-7: SOS Cytosensor Strengths and Weaknesses Chart

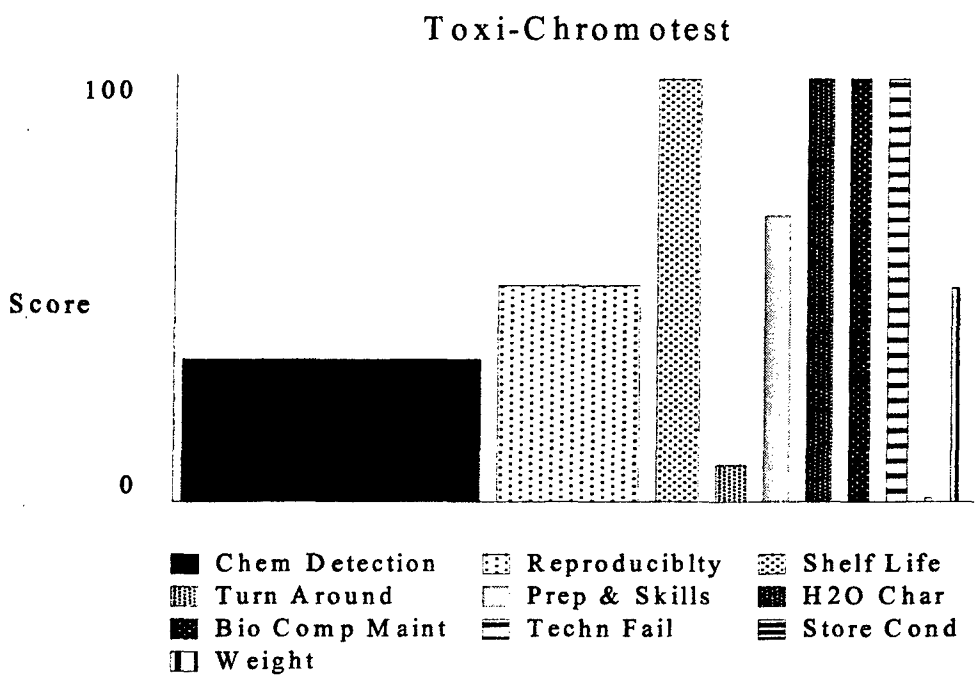

Figure K-8: Toxi-Chromotest Strengths and Weaknesses Chart

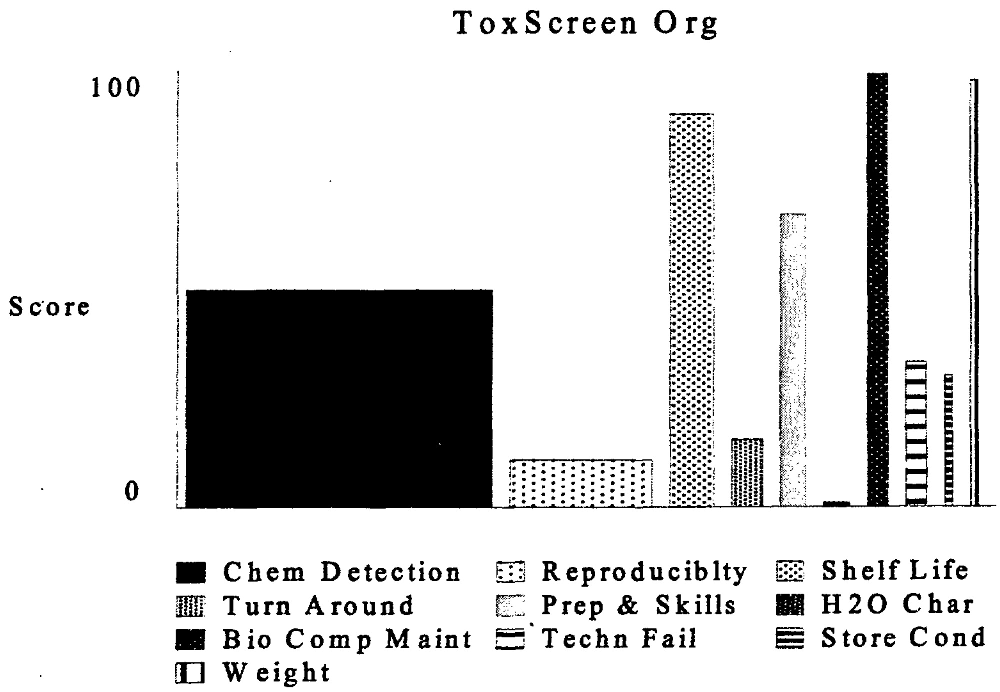

Figure K-9: ToxScreen Organic Strengths and Weaknesses Chart

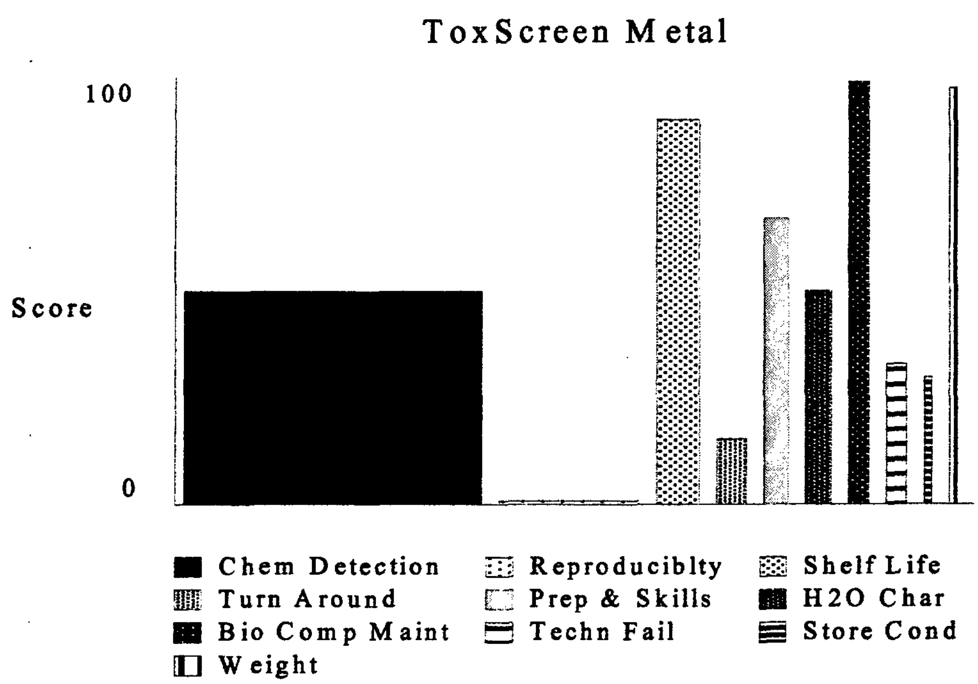

Figure K-10: ToxScreen Metal Strengths and Weaknesses Chart

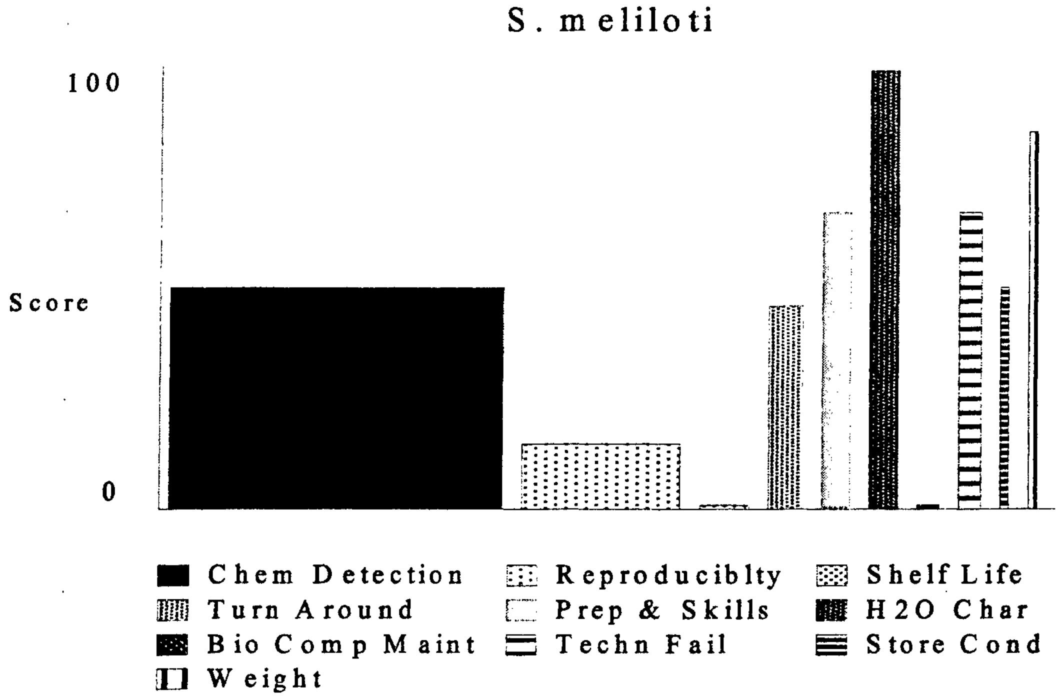

Figure K-11: *Sinorhizobium meliloti* Assay Strengths and Weaknesses Chart

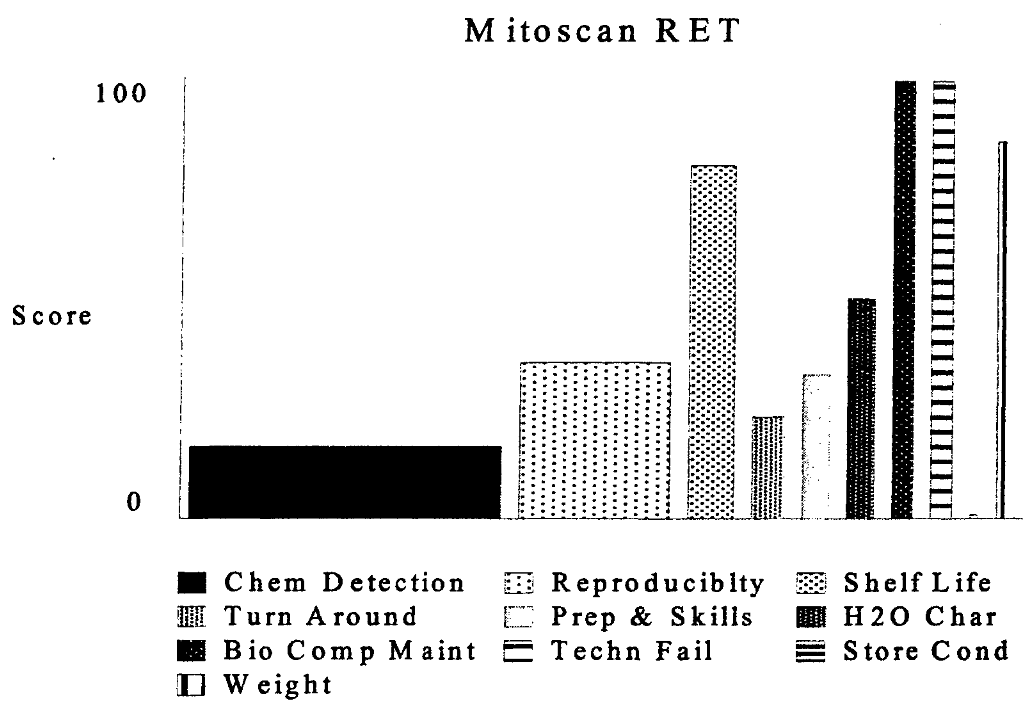

Figure K-12: Mitoscan Reverse Electron Transfer Strengths and Weaknesses Chart

APPENDIX L

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM: QUANTITATIVE MODEL MEASURES ANALYSIS

The measures analysis summarizes the assessment results in terms of individual measures. Because the measures represent user needs, this analysis helps identify areas of shortfall, or potential technical challenges (e.g., if the ESB technologies generally score low against a measure). Conversely, the analysis also identifies areas of minimal concern (e.g., the technologies generally score high and/or the measure is low-weighted).

The range of scores for each measure was examined to determine overall ESB technology performance relative to each measure, which provided the basis for assignments of a subjective assessment rating by the DAT. Comments and rationale for each measure were also provided. Note that all measures were evaluated as either a green or yellow rating. *Table L-1* summarizes the results from the measure analysis along with defining subjective ratings.

Table L-1: Measure Analysis

Measures (Weight – see Note 1)	**Rating** (see Note 2)	**Comments**
Chemical Detection (0.49)	Green (see Note 3)	- This measure is weighted the highest, almost half of the models weight. - The utility function is a linear continuous curve. For each chemical detected the technology earns 16.7% of the possible score. For example, detecting four chemicals would earn a score of 66.8 (4 x 16.7) out of 100. - Seven technologies detected three chemicals or fewer and thus earned half or less of the possible maximum score.
Water Characteristics (0.04)	Green	- This measure is weighted very low and thus has a small overall impact on the models score. - The utility function is four discrete levels. In this function it is important to be able to operate in water with at least 1 mg/L of residual chlorine because at least half of the total possible score is earned by doing so. - Six technologies scored at the highest level for the measure (i.e., they could operate in water with over 5 mg/L of residual chlorine), three scored at the 50% point (i.e., can handle residual chlorine between 1-2 mg/L only), and three scored at the lowest level (i.e., they could operate in water with less than 1 mg/L of residual chlorine). - Dechlorinating the water before using the ESB technology can mitigate issues with residual chlorine.

Technology Failure Rate (0.03)	Green	- This measure is weighted very low and thus has a small overall impact on the models score. - The utility function is four discrete levels. The possible range of failure rates range from 0-15+% with any rates between 5-15% earning a relatively low 33 out of 100 score. - Results for this measure were determined from controlled experiments. - Five technologies scored at the highest level (i.e., 0% failure rate). Neuronal Microelectrode Array is the only technology that scored a zero for this measure (i.e., more than 15% failure rate). Technical Experts felt this failure rate could be significantly reduced by fixing know shipping problems.
Test Reproducibility (0.23)	Yellow	- This measure is weighted the second highest, almost a quarter of the models weight. - The utility function is a non-linear continuous curve. Having a median coefficient of variation of 20% earns about 30 out of 100 score. Having a median coefficient of variation of 12% earns about a 70 out of 100 score. There is a sharp increase in score for earning a median coefficient of variatiòn of greater than 20%. - Two technologies scored very well (Eclox and Mitoscan Electron Transfer), four scored very low (SOS Cytosensor, ToxScreen Organic, ToxSceen Metal, and *Sinorhizobium meliloti* Assay), and the rest scored in the middle.
Test Turn Around Time (0.05)	Yellow	- This measure is weighted low and thus has a small overall impact on the models score. - The utility function is a non-linear continuous curve. It is much more important to score closer to 5 minutes than 180 minutes. A turn around time of 20 minutes earns a score of 75 out of 100 score while 40 minutes earns 40 out of 100 score and 140 minutes earns less than 5 out of 100 score. - Nine technologies scored less than 25 out of 100.
Preparation and Skills Required (0.04)	Green	- This measure is weighted very low and thus has a small overall impact on the models score. - The utility function is four discrete levels. The lowest level scores 0 out of 100. This lowest level requires many sample preparation steps and/or expert skills to prepare the sample. The other three levels are each worth 1/3, 2/3, and 100% of measures score. - Two technologies scored a 0, Neuronal Microelectrode Array and SOS Cytosensor, which means the skills of a research scientist with a lab are required.
Weight (0.01)	Green	- This measure is tied with the *Storage Conditions Required* measure as the lowest weighted measures and thus has a small overall impact on the models score. - The utility function is a non-linear continuous curve (backwards s-shape). It is much more important to score closer to 1 lb vs. 40 lbs. For example, a technology weighting 10 lbs earns a score of 85 out of 100, 20 lbs earns a 50, and 30 lbs earns a 10. - Six technologies scored above 70, two score between 40-50, and three scored below 5 out of 100.

Shelf Life (0.07)	Yellow	- This measure is weighted low and thus has a small overall impact on the models score. The utility function is a non-linear continuous curve. Although, it is important to have a shelf life for 12-months it is most important to have a shelf life of at least 3-months as this earns a score of 50 out of 100 and 6-months earns an 80. - Five technologies have a shelf life of one month or less. All other technologies have a shelf life of longer than six months.
Storage Conditions Required (0.01)	Yellow	- This measure is tied with the Weight measure as the lowest weighted measures and thus has a small overall impact on the models score. - The utility function is four discrete levels. The average level, which is defined further as controlled room temperature, earns 50 out of 100. The two levels below average earn a 30 and 0 out of 100. Thus any technology requiring any temperature control scores low for this measure. - Seven technologies scored at the lowest level. Only one technology, Eclox, scored at the highest level and one scored at the average level – SOS Cytosensor.
Biological Component Maintenance (0.03)	Green	- This measure is weighted very low and thus has a small overall impact on the models score. - The utility function is three discrete levels. If a technology requires any culture medium exchange it scores 0 out of 100. - Nine technologies scored at the highest level but two at the lowest level meaning the technology requires culture medium exchange or CO2 for culture medium.

Note 1: Weights (e.g., 0.49 for *Chemical Detection* measure) is for the Fixed Base Scenario model.

Note 2: Rating Key:

Green: Area of low concern; indicating user needs should be met, or the measure is low weight and is unlikely to cause significant impact.

Yellow: Area of moderate concern; indicating user needs may not be met.

Red: Area of high concern; indicating user needs are not readily met.

Note 3: Because the maximum number of chemicals detected by any technology is six this is the 100 score. It is a goal to detect all twelve chemicals, though. In order to detect more than six chemicals an ESB technology system is needed (i.e., two or more technologies working together).

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APPENDIX M

ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM: MEASURE SENSITIVITY ANALYSIS CHARTS

Sensitivity analysis was also performed to determine whether changes in measures weights would affect the results. This analysis was conducted on all measures. LDW was used to generate sensitivity graphs for each measure. The sensitivity graphs contain the overall score on the y-axis (from worst to best) and the percent of the weight given to the measure being evaluated on the x-axis (from 0 to 100). There is a line segment for each ESB technology on the graph, which extends from the x-axis at various angles to the position that corresponds with 100% of the weight being assigned to that measure. A vertical line on the sensitivity graph identifies the current weight assigned to the measure. The order in which the vertical weight line intersects the alternatives' segments is the overall rank order produced by the model's weighting scheme.

By visually noting the changes in rank order which would occur if the vertical weight line was moved to the right or left (measure weight increased or decreased), an assessment can be made as to the sensitivity of the weight of that measure. If a slight movement of the weight line causes multiple alternative lines to cross (and therefore the rank order to have multiple changes), then that measure would be considered very sensitive to weight changes. If, on the other hand, no ranking changes occur when the line is moved a greater distance, then it can be determined that the weight of that measure does not have a notable effect on the outcomes of the analysis.

For the most part, the technology rankings are insensitive to reasonable (± 15% changes) measure weight changes. This is especially true for the top three scoring technologies (Microtox, Hepatocyte Low Density Lipoprotein Uptake, and Electric Cell-Substrate Impedance Sensing). There are two instances were reasonable weight changes have an impact:

- The first instance is when the *Chemical Detection* measure weight is increased or reduced 15% (considered maximum reasonable change; see *Figure M-1)*. By decreasing the weight of the *Chemical Detection* measure 15% Mitoscan Electron Transfer and Eclox change positions in the ranking (3rd and 4th positions). Toxi-Chromotest increases from 8th highest ranked to 6th highest ranked. By increasing the weight of the *Chemical Detection* measure 15%, the impact on the ranking is a little less as only SOS Cytosensor increases from 7th highest ranked to 5th highest ranked and exchanges ranking with Eclox.
- The second instance is when the weight of the next highest weighted measure, *Test Reproducibility*, decreases 15% (see *Figure M-4*). When this happens, SOS Cytosensor increases from seventh highest ranked to fifth highest ranked. Eclox and Neuronal Microelectrode Array each drop one position in ranking. When the measure is increased 15% there is only a slight impact - Electric Cell-Substrate Impedance Sensing and Mitoscan

- Electron Transfer would rank the same and SOS Cytosensor and Toxi-Chromotest would rank the same.

Sensitivity analysis was conducted for all ten-evaluation measures for all three models/scenarios. Sensitivity graphs for the measures for the Fixed Base Scenario are provided in *Figures M-1 – M-12*.

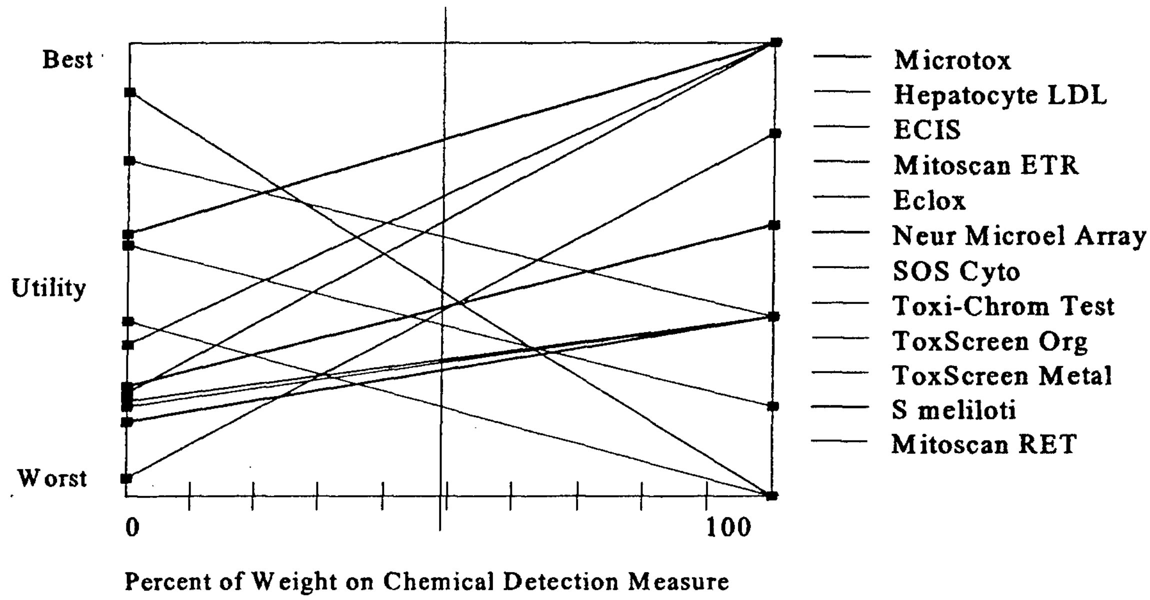

Figure M-1: *Chemical Detection* Measure Sensitivity Chart

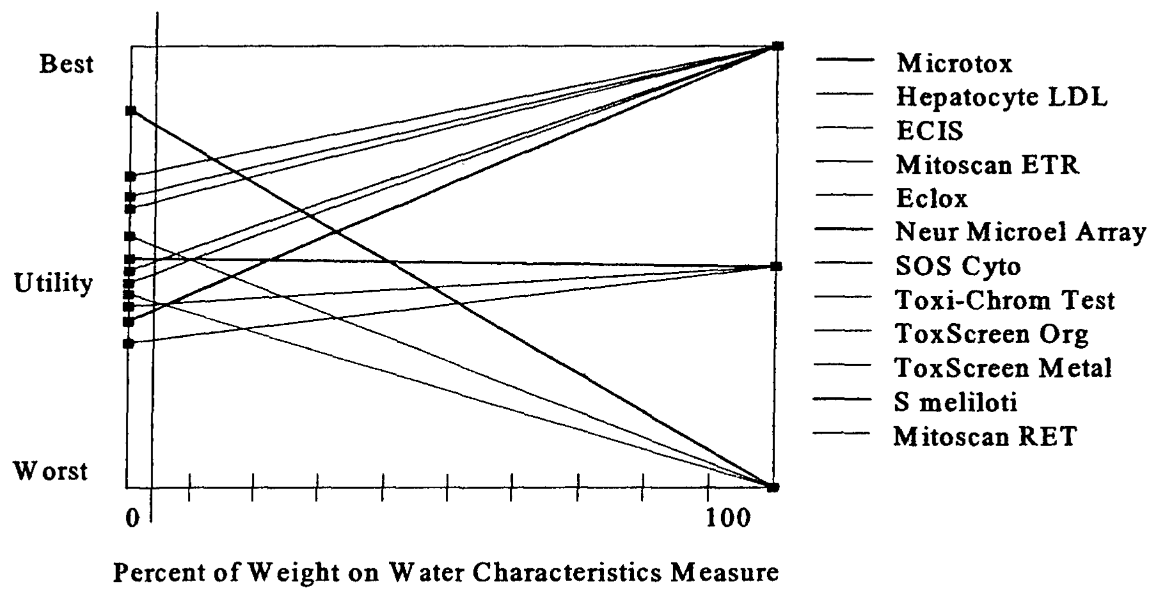

Figure M-2: *Water Characteristics* Measure Sensitivity Chart

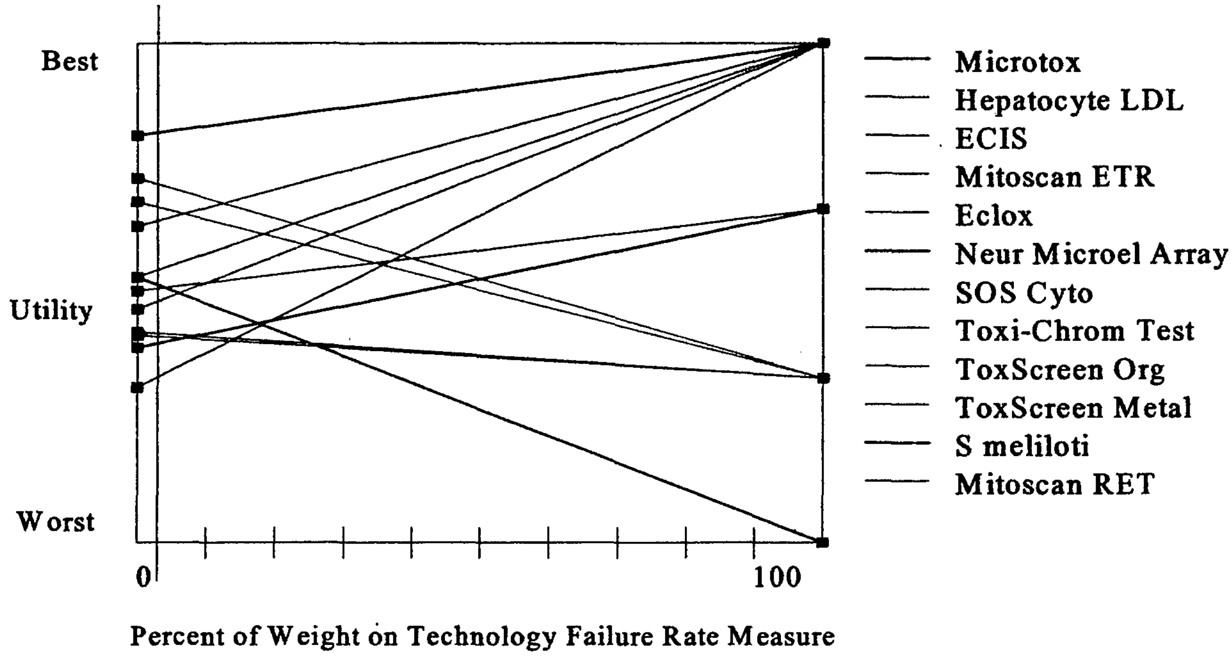

Figure M-3: *Technology Failure Rate* Measure Sensitivity Chart

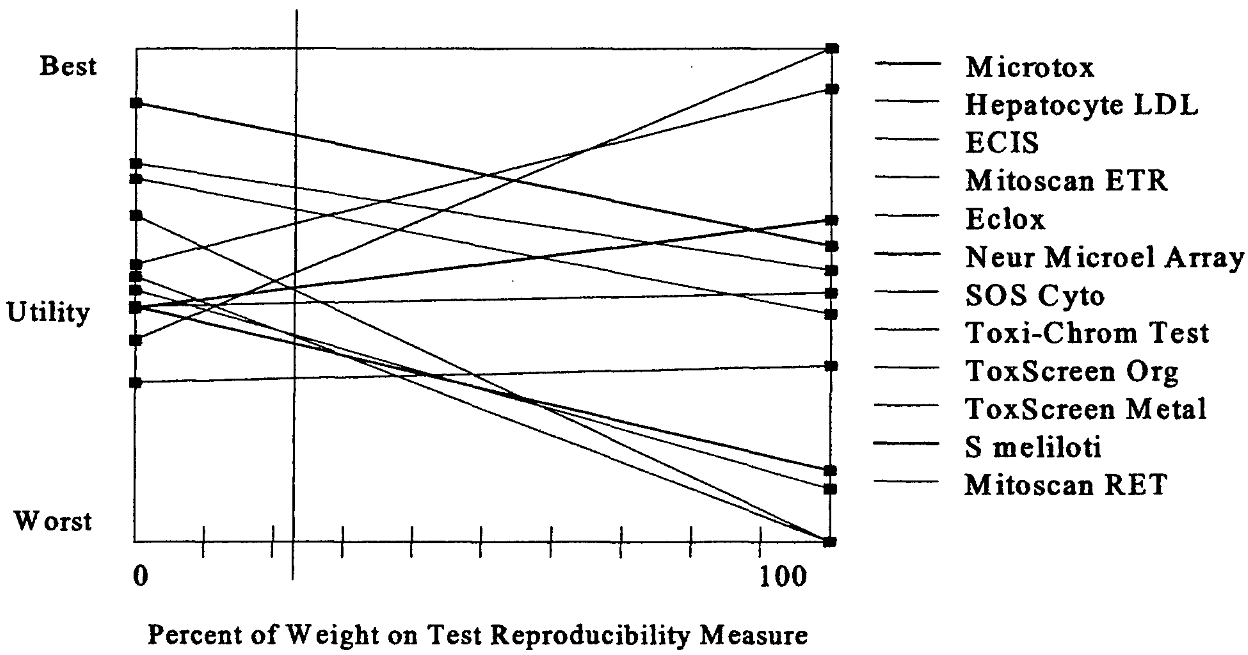

Figure M-4: *Test Reproducibility* Measure Sensitivity Chart

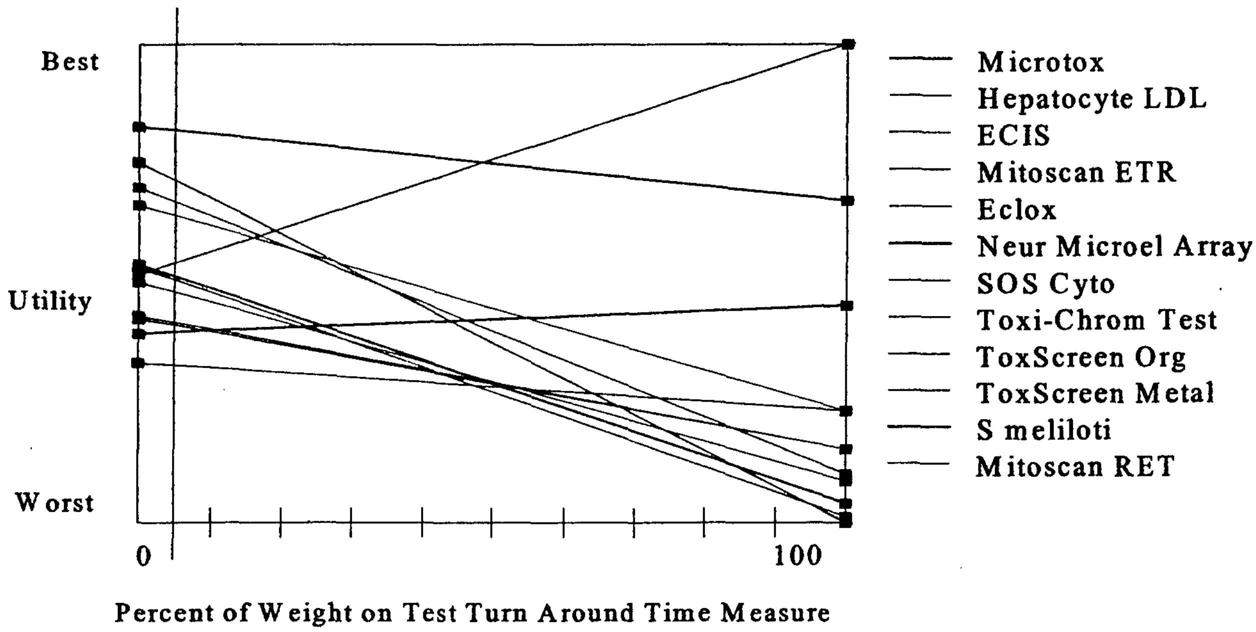

Figure M-5: *Test Turn Around Time* Measure Sensitivity Chart

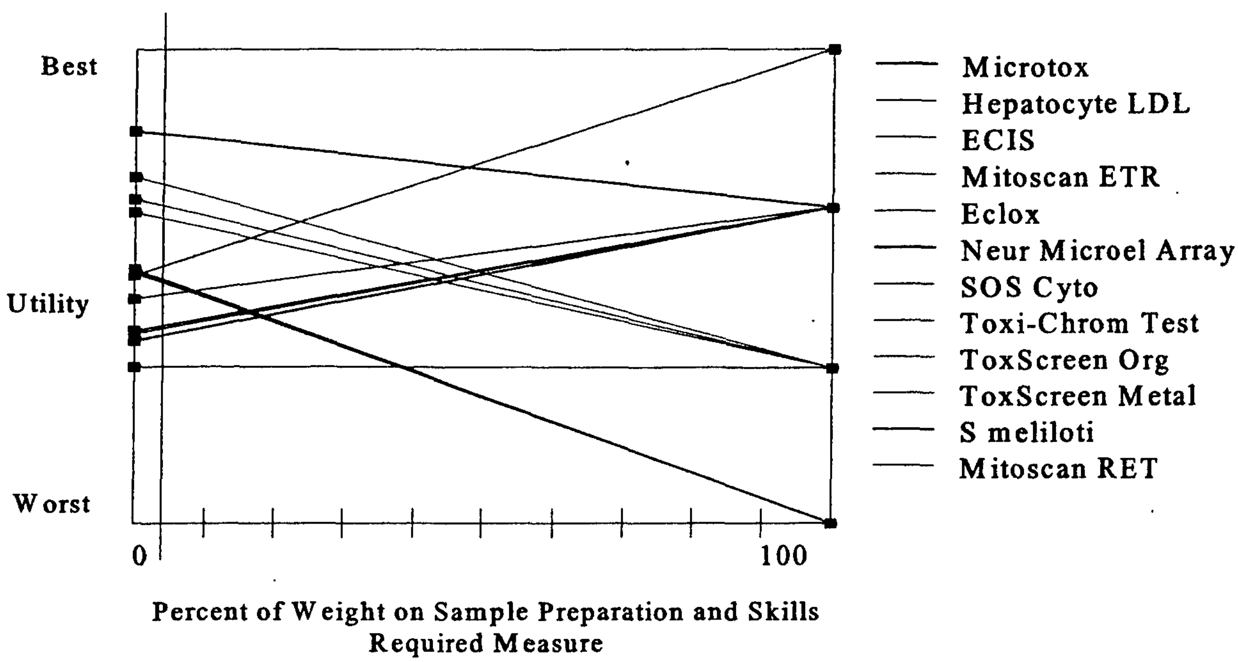

Figure M-6: *Sample Preparation and Skills Required* Measure Sensitivity Chart

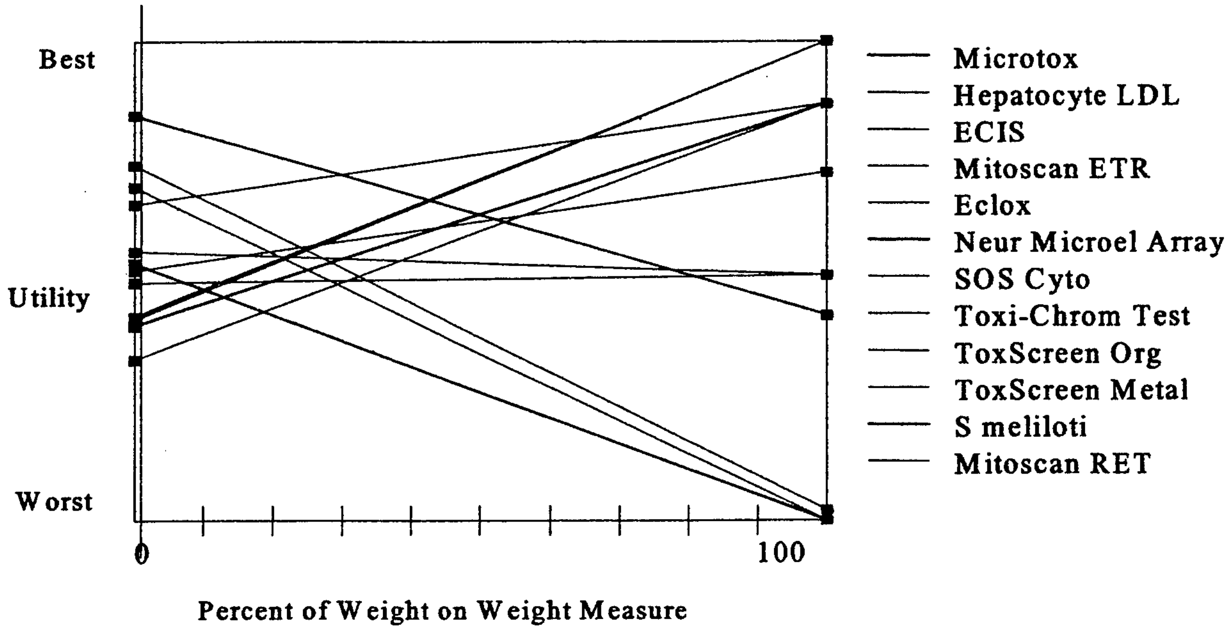

Figure M-7: *Weight* Measure Sensitivity Graph

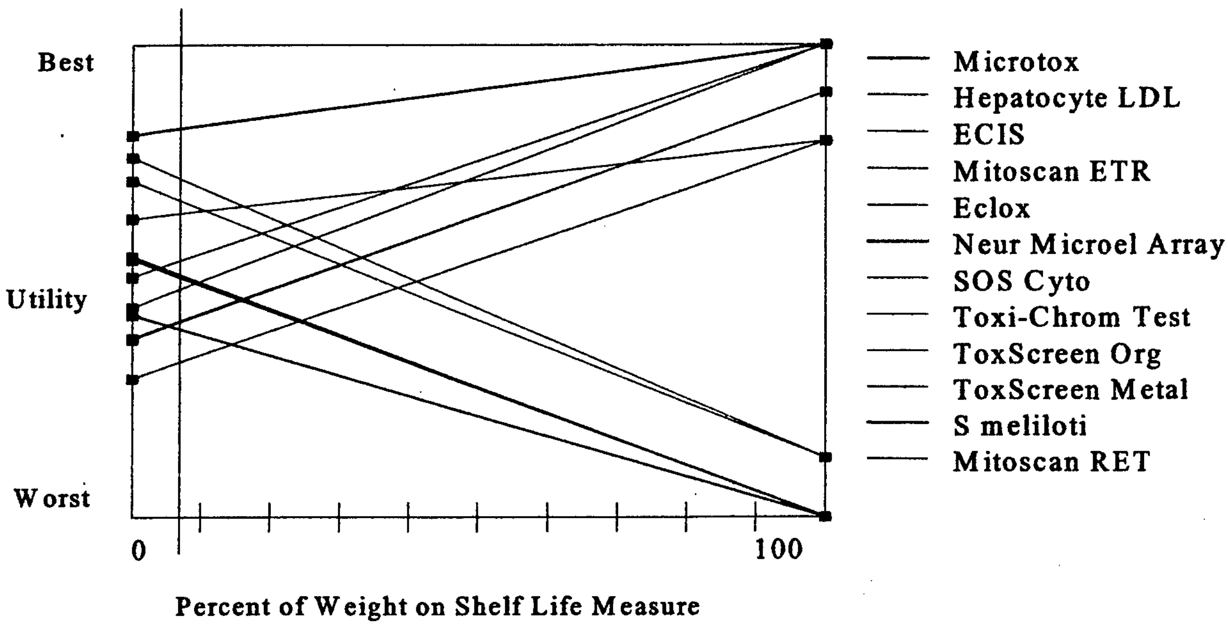

Figure M-8: *Shelf Life* Measure Sensitivity Chart

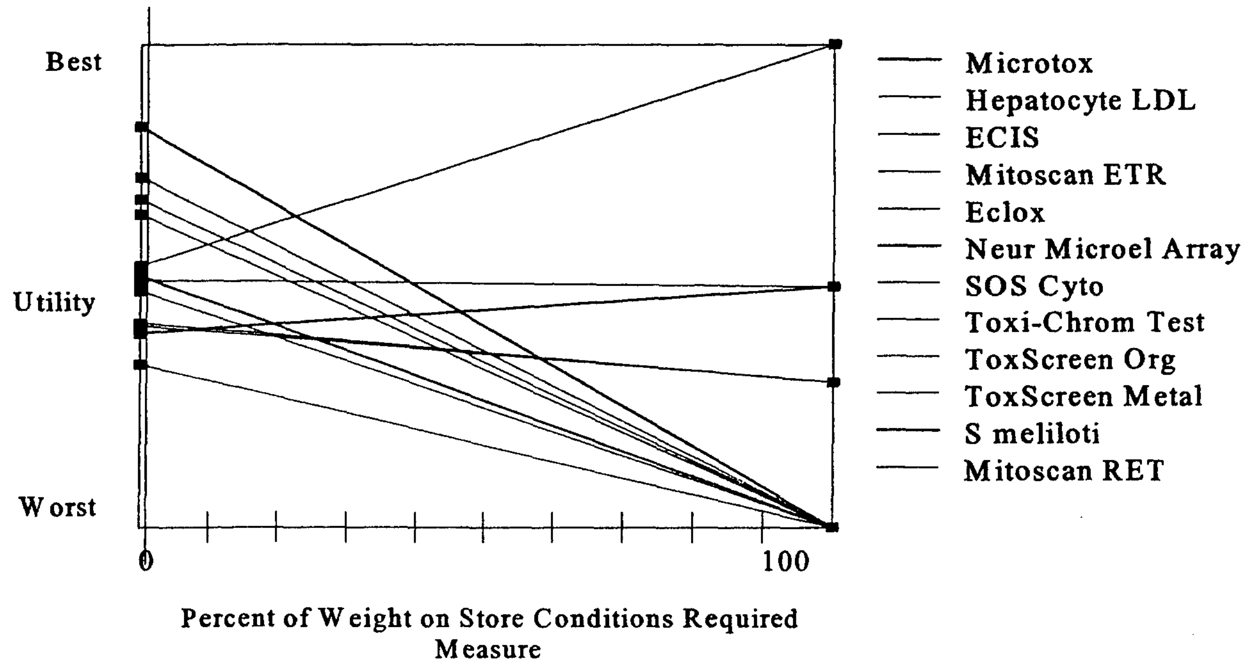

Figure M-9: *Storage Conditions Required* Measure Sensitivity Chart

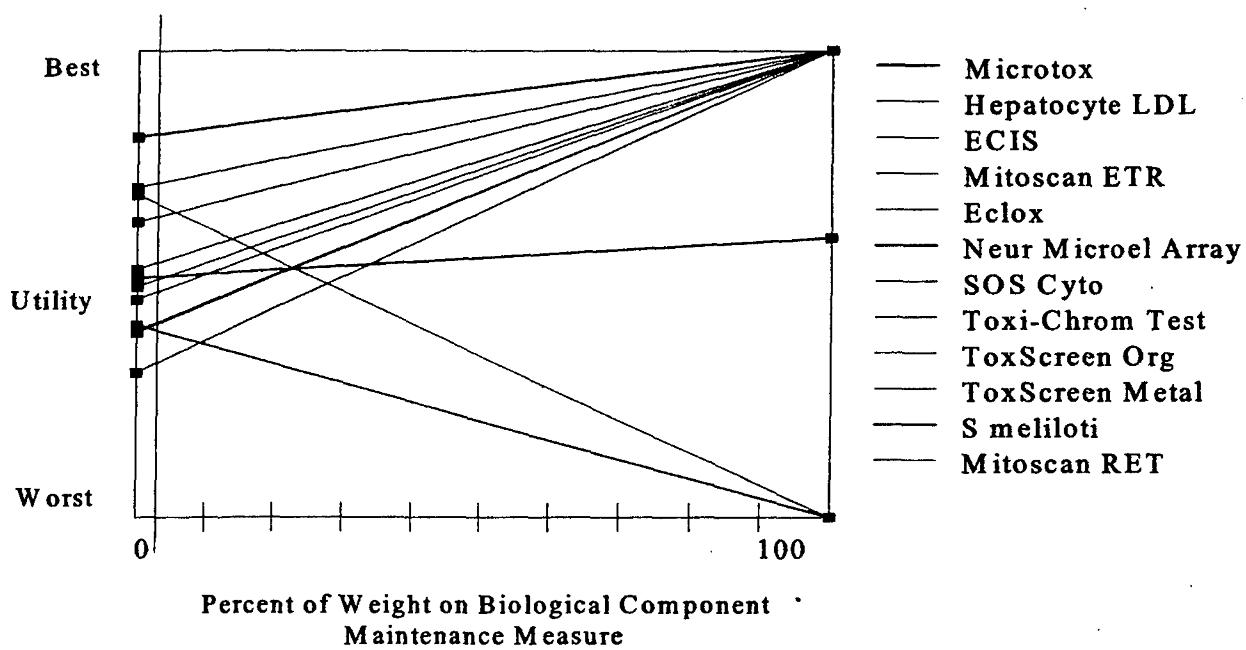

Figure M-10: *Biological Component Maintenance* Measure Sensitivity Chart